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Trophic state of the Great Masurian Lakes system in the past, present and future

- causes, mechanisms and effects of changes

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ABSTRACT

Background

Lakes of the Great Masurian Lakes system (GML-system) have been the topic or model ecosystem in a large amount of studies, but a look at the recent history of ecological changes and evaluation of the present ecological state have not been previously performed in a comprehensive way.

For this purpose, I utilised a database of published and unpublished ecological data from 1980-2008 collected by the Microbial Ecology Department. In addition, the present state of the GML-system was investigated during the spring and summer months of 2009, 2010 and 2011. These datasets were combined to evaluate changes in water quality from 1980 onwards. The ecological changes were compared to external input data from a governmental statistical office. The present ecological state of the system was analysed in more detail, and the best strategies to further decrease the trophic state of the lakes and to limit the biomass of phytoplankton and especially cyanobacteria were investigated.

Results and conclusions

The external nutrient inputs to the GML-system began to decrease in 1990. Signs of oligotrophication became clear after 1995 and continued up to 2005. The highest improvement occurred in Lake Niegocin, owing to highly improved wastewater treatment in the town of Giżyczko. After 2005 the oligotrophication of the GML-system stopped, which corresponds to increasing nutrient inputs from agriculture and tourism and possibly also from internal sources.

The differences in trophic state index (TSI) between lakes have decreased significantly. During 2009-2011, mean TSIs ranged from 50 to 63, i.e. all lakes could have been classified as

eutrophic. The trophic state of the GML-system was highly variable and dependent on weather conditions. In 2010, an extreme heat wave caused significant changes in multiple variables (such as limnetic nutrient concentrations and stratification structure) and possibly internal loading of phosphorus from sediments to epilimnion.

Main effects of eutrophication of the GML-system were identified to be increased dominance of primary production over respiration (i.e. accumulation of organic matter), stronger nitrogen limitation and increased biomass of cyanobacteria. Phytoplankton growth was not found to be limited by a single nutrient, but rather showed co-limitation by nitrogen and phosphorus. Still the conducted analyses supported the classical view that decreasing external phosphorus inputs to lakes is the best lake management approach for controlling eutrophication and biomass of cyanobacteria. Possible solutions for decreasing external phosphorus inputs to the GML-system are, for example, the construction of vegetative riparian buffers and the improvement of wastewater treatment efficiency, especially in Ryn and Mikołajki.

ABSTRAKT

Wprowadzenie

Jeziora systemu Wielkich Jezior Mazurskich (GML) były obiektem wielu opracowań i modelowych studiów ekologicznych. Jednakże jedynie bardzo nieliczne z nich dokumentowały ewolucję ich trofii w kontekście historycznym i w sposób kompleksowy analizowały przyczyny i skutki zmian w ich zlewniach jakie ukształtowały obecny stan troficzny zarówno poszczególnych jezior jak i całego systemu GML.

W celu takiej analizy autorka wykorzystała głównie publikowane jak i niepublikowane wyniki badań z lat 1980-2008 zgromadzone przez Zakład Ekologii Mikroorganizmów Uniwersytetu Warszawskiego, raporty i opracowania Stacji Terenowej PAN w Mikołajkach, nieliczne inne dostępne dane literaturowe oraz wyniki badań własnych przeprowadzonych w sezonach wiosenno-letnich, w latach 2009-2011. Analizując zmiany w ekologii badanych jezior tak skonstruowaną bazę wynikową skojarzono z danymi Głównego Urzędu Statystycznego, pozyskanymi w miejskich i gminnych oczyszczalniach ścieków w Giżycku, Rynie oraz z raportami lokalnych agend ochrony środowiska dotyczącymi zmian tempa zewnętrznego dopływu biogenów.

Bardziej szczegółowo przeanalizowano obecny stan troficzny jezior system GML wskazując optymalne strategie dla podtrzymania ich oligotrofizacji, skutkującej zmniejszeniem się w ich wodach ogólnej biomasy fitoplanktonu oraz dominacji nitkowatych Cyanobakterii uciążliwych dla środowiska i gospodarki człowieka. Procesy szybkiej oligotrofizacji południowej części system GML obserwowano zwłaszcza w latach 1995-2005.

Wyniki i wnioski

Systematyczny spadek tempa zewnętrznego dopływu biogenów do systemu GML rozpoczął się w połowie lat 90-tych ubiegłego wieku. Pierwsze oznaki oligotrofizacji jezior tworzących jego część południową obserwowano już w roku 1996. Procesy skutkujące obniżeniem się ich statusu troficznego ulegały systematycznemu przyspieszeniu do roku 2005. Ich najbardziej spektakularne skutki ujawniły się w jeziorze Niegocin po wybudowaniu w mieście Giżycko nowoczesnej oczyszczalni ścieków. Zmniejszenie się ładunku biogenów dopływającego do wód Niegocina, będącego jednym z punktów kluczowych systemu GML, pociągnęło za sobą również zmniejszenie się skali transportu biogenów do jezior leżących poniżej i w ten sposób uruchomiło procesy ich oligotrofizacji. Po roku 2005 tempo oligotrofizacji południowych jezior systemu GML uległo wyhamowaniu, co zbiegło się w czasie ze wzrostem zewnętrznego dopływu biogenów generowanego przez odtwarzające się w szybkim tempie wielkoobszarowe rolnictwo oraz ciągle zwiekszajacy się ruch turystyczny. W przypadku niektórych jezior istotne znaczenie miał także prawdopodobnie ciągle ilościowo istotny wewnętrzny dopływ fosforu I azotu z osadów dennych.

W latach 2009-2011 obserwowano zminejszenie sie różnic pomiędzy indeksami stasusu troficznego (TSI) jezior systemu GML. Średni TSI wyliczony dla każdego z jezior systemu whał się w tym okresie od 50 do 63, co oznacza, że każde z nich można było sklasyfikować jako eutroficzne. Jednakże w poszczególnych latach TSI większości z badanych jezior charakteryzował się znaczną zmiennością zależną od warunków pogodowych. W roku 2010 fala upałów spowodowała istotne zmiany fizycznej struktury słupa wody oraz zasięgu i miąższości poszczególnych stref wód głębokich jezior rynnowych. Spowodowało to prawdopodobnie wzrost tempa uwalniania biogenów z ich osadów dennych do wód profundalu a skrócony dystans pomiędzy odtlenionym hypolimnionem i strefą limnetyczną umożliwił ich efektywne podsiąkanie do wód powierzchniowych, a w efekcie, udokumentowany uzyskanymi wynikami, okresowy wzrost stężenia biogenów oraz tempa produkcji pierwotnej i biomasy fitoplanktonu.

ABSTRAKT

Głównymi efektami eutrofizacji jezior systemu GML były: powiększanie się różnicy pomiędzy tempem produkcji pierwotnej a tempem respiracji, prowadzący do akumulacji materii organicznej w wodach jeziornych, narastający deficyt dostępnych form azotu oraz wzrost biomasy cyjanobakterii. Tempo wzrostu i rozwój zespołów fitoplanktonowych nie były przy tym limitowane niedostatkiem jednego z biogenów lecz były raczej efektem ko-limitacji azotowo - fosforowej.

Wszystkie uzyskane wyniki potwierdziły klasyczny pogląd, że najlepszym sposobem kontroli jakości wód jeziornych oraz tempa procesów eutrofizacyjnych jest ograniczenie zewnętrznego dopływu fosforu do wód jeziornych. W przypadku jezior systemu GML optymalnym sposobem spowolnienia procesów eutrofizacyjnych, podtrzymania procesów oligotrofizacji oraz ograniczenia biomasy cyanobakterii jest ścisła ochrona istniejacych oraz tworzenie, tam gdzie to możliwe, roślinnych stref buforowych (vegetative riparian zones) wokół jezior, utrzymanie wysokiej efektywności usuwania biogenów przez oczyszczalnię ścieków w Giżycku oraz znaczącą poprawę sprawności oczyszczalni w Rynie i w Mikołajkach.

ABBREVIATIONS AND DEFINITIONS

Abbreviations

- AMP: L-leucine aminopeptidase
- APA: alkaline phosphatase
- Chl *a*: chlorophyll *a*
- DOC: dissolved organic carbon
- GML-system: Great Masurian Lakes system
- GPPr: gross primary production rate
- Lowess: locally weighted scatterplot smoothing
- No: number
- NPPr: net primary production rate
- PER: percentage extracellular release
- Rr: respiration rate
- SD: standard deviation
- SE: standard error
- TN: total nitrogen
- TN:TP ratio: ratio between total nitrogen and total phosphorus (in weight)
- TP: total phosphorus
- TSI: trophic state index

Common definitions

- Zones by light gradient

  Limnetic zone: well-lit, open surface area of a body of water, away from shore. Light intensity is at least 1% of the maximum light intensity at the surface.
  Profundal zone: zone beyond effective light penetration (light intensity less than 1% of the maximum at the surface of a body of water); here used synonymously with zones below surface waters (metalimnion and hypolimnion together).
  Littoral zone: an area near shore, where light penetrates the whole water column to the sediments and allows aquatic macrophytes and epipelic algae to grow (light intensity at the sediment surface at least 1% of the maximum at the surface of water).

- Zones by temperature gradient

  Epilimnion: upper layer of a body of water, where water is mixed and temperature change is less than 1°C per metre of depth.
  Metalimnion = thermocline: a layer in a body of water which separates regions differing in temperature and where temperature change is more than 1°C per metre of depth.
  Hypolimnion: lowest water layer of a body of water, which lies below thermocline.

- Average

  Arithmetic mean value

Specific definitions

- Trophic state index
  An index value describing the "nutrient" state of a body of water. Calculated as a mean value of indexes based on TP, chl *a* and Secchi disk visibility according to Carlson (1977).
- Eutrophication
  Increase in rate of supply of organic matter to an ecosystem, which leads to changes in the ecosystem and impairment of human use of the ecosystem's services.
- Oligotrophication
  Decrease in rate of supply of organic matter to an ecosystem, which improves the value of the ecosystem for human usage.
- Hypoxia
  Reduced oxygen content of water; commonly defined as oxygen saturation ranging from 1% up to 30%. Here, hypoxia is defined as a range of oxygen concentrations ranging from 0% oxygen saturation (i.e. full anoxia) to a maximum of 25%.
- Oxygenated buffer zone
  A zone separating hypoxic water from epilimnion. It is characterized by an oxycline from full saturation near epilimnion towards hypoxia in meta- or hypolimnion (i.e. oxygen saturation ranging from 100% down to 25%).
- Vegetative riparian buffer
  Roughly 20 metre wide strip of trees or bushes, which separates agricultural land from lake water.

INTRODUCTION

The Great Masurian Lakes system (GML-system) has been a centre of human habitation for centuries. Local activities of the denizens - spanning from expanding settlements and cutting down forests to agriculture and tourism - have profoundly influenced its functioning and ecology. Intensification of anthropogenic pressure during the last hundred years caused the Masurian lakes to go through a phase of fast eutrophication. Eventually, political changes and concern over the state of the environment lead to a decrease in the amount of nutrients discharged into the lakes about 20 years ago. This allowed the lakes to begin their ecological recovery. During the last ten years, it appears that the anthropogenic pressures are rising again: agriculture and especially tourism are becoming more intensive. The anthropogenic pressures have profoundly modified the Masurian lake ecosystem and their present ecological state cannot be fully understood without knowledge of the past and present. Without a full understanding of the past and present it will also be impossible to predict what threats or positive developments might arise in the future. As a high trophic state adversely influences various aspects of biological diversity, physico-chemical factors and flows of energy and matter in all lakes of the GML-system, eutrophication is the most serious threat for the lakes. The risk that eutrophication might be once again occurring should be evaluated, the perpetrators identified and the new nutrient sources should be in some way compensated for or minimised.

The eutrophication of lakes was an international problem during the 20th century; its causes and consequences have been reported in depth (for example Rast and Holland 1988, Schindler 2006, Smith and Schindler 2009). Despite extensive research, there are still some less understood factors related to eutrophication: for example, interplay with ecosystem stability, pathogens and other non-nutrient contaminants. In comparison, oligotrophication of lakes is a relatively new large-scale phenomenon and there is a serious lack of information about its effects

(outside the obvious lowering of trophic status) and how it influences ecosystems. It seems clear that an ecosystem recovering from a disturbance does not follow the same trajectory it took during the time when the disturbance was occurring (Duarte et al. 2009). However, how these trajectories differ and what this means for an ecosystem are largely unknown.

Generally, oligotrophication is not always achievable, even when external inputs are decreased severely (Carpenter et al. 1999). Many factors can disturb recovery and cause a need for restorative measures (for example internal loading and piscivore density, Carpenter and Kitchell 1988, Sorrano et al. 1997). Other possible problems which can prevent oligotrophication should also be identified and the risks related to them evaluated.

Eutrophication and nutrient cycles in lakes

The trophic state of a lake ecosystem has been given various definitions in the literature. The main difference between the main groups of definitions is whether eutrophication is given a negative association due to its impact on humans. Strictly without such association, eutrophication can be defined as a sum of various inputs and outputs of nutrients or as an increase in the rate of supply of organic matter (Nixon 1995). Inputs can originate from external point and nonpoint sources or from internal sources. The external point inputs are municipal wastewater treatment plants, rain water sewers, industrial sources and inputs from other lakes or rivers. External nonpoint sources include natural background sources from the erosion of rocks, leakage from natural terrestrial environments and also inputs from agriculture, scattered settlements and from the atmosphere (through rainfall or dust). Internal inputs have two main routes: recycling of nutrients within the water column and release of nutrients from sediments. Outputs of nutrients are, for example, outflows of water and sedimentation. These various fluxes and pools of nutrients can also be modified by the community living in the ecosystem: for example some fishes can, through their habit of digging food from the bottom of a lake, increase the release of nutrients from the

sediments; and cyanobacterial N_2-fixation and bacterial denitrification and anammox processes can also modify the nitrogen pool. In this sense, eutrophication is seen as either a natural ageing process of lakes or as an enhancement of the natural process caused by anthropogenic nutrient influx. Another type of definition has been suggested by OECD (1982): "eutrophication is the process of nutrient enrichment of waters which results in the simulation of an array of symptomatic changes, amongst which increased production of algae and aquatic macrophytes, deterioration of water quality and other symptomatic changes are found to be undesirable and interfere with water uses". This concept adds the idea of the reduced value of freshwater resources for human use, which is missing from other definitions. This is the view followed here.

Lakes naturally go through changes in the inputs and outputs of nutrients. Over centuries, lakes gather inputs from the surrounding landscape and atmosphere and accumulate some of these inputs, which lead to a slow increase in their trophic states. This natural process of eutrophication eventually leads to overgrowing of the lake as it fills up with organic matter and vegetation. Eventually, the lake turns into a marsh and ultimately becomes a terrestrial habitat (Weber 1907). This view of the “slow natural eutrophication process of lakes” might in some cases be too simplistic, but it generally probably quite correctly describes the ecological history of temperate lakes which were formed after the ice age.

Humans have greatly altered the nutrient cycles of lakes, especially since the agricultural and industrial revolutions. An increase in the human population, the usage of industrial mineral fertilisers and the change from a closed anthropogenic nutrient cycle (where human and animal faeces and organic waste are used as fertilisers on fields) to an open-ended anthropogenic nutrient cycle (where waste is deposited on landfills or discharged into rivers or lakes) have greatly increased the volume of external inputs to aquatic ecosystems. The anthropogenic inputs commonly make up the clear majority of total nutrient inputs to aquatic ecosystems and have caused great changes in the ecology and functioning of these ecosystems. Consequently, the current ecological

state of a human influenced aquatic ecosystem cannot be understood or its future predicted without looking at past and present anthropogenic inputs and the history of trophic state changes.

In addition to the external sources, internal cycles are an important source of nutrients. The internal sources of nutrients (mainly regeneration and internal loading) are much more difficult to measure and to understand than external inputs. Recirculation of nutrients can have different routes – for example bacterial mineralisation and the release of nutrients through grazing. The nutrients in DOM can be reutilised by bacteria and other organisms through enzymatic reactions which breakdown the larger molecules into smaller products ready for uptake by microbial organisms (Chróst 1991). Utilisation and reactivity of DOM has been estimated indirectly as increased bacterial biomass (Søndergaard et al. 1995), as the bacterial production rate (Chróst et al. 2000) or directly as the fraction of DOM which can be utilised by bacteria (Coffin et al. 1993). Similar methods also exist for the estimation of phosphorus regeneration from DOM: the enzymatically hydrolysable phosphorus-method by Chróst et al. (1986) and the radiophosphorus method by Hudson and Taylor (1996). Results obtained from these methods show that, for example, the recycling rate of phosphorus within the water column can range from less than 0.001 up to 6 ng-P L^{-1} h^{-1} (Hudson et al. 1999, Gao et al. 2006). Similar methods for measuring nitrogen regeneration within the water column are still lacking.

The second major internal source of nutrients is the release of nutrients from sediments. Both nitrogen and phosphorus can be released from the sediments through multiple routes (Katsev et al. 2006, Özkundakci et al. 2011). Anoxia greatly enhances, in particular, phosphorus release through the unbinding of phosphorus from iron complexes. The released nutrients can then escape to overlaying water. Lakes with intensive internal cycling of nutrients are generally shallow (more than half of lake area less than 5 metres deep), have alkaline pH (which decreases the chance of phosphate being absorbed to clays and oxides; Reynolds 2003) or have high amounts of sulphate (can cause the inhibition of binding between phosphorus and iron; Katsev et al. 2006). The fate of

phosphorus depends on multiple factors, especially in deep lakes (Nowlin et al. 2005). The best known routes of internal phosphorus loading occur during spring and autumn overturns when the nutrients are mixed into the whole water column and thus create seasonal internal fluxes of nutrients. During summer months, stratification can inhibit the flux of nutrients from sediments to epilimnion if part of the profundal zone remains oxygenated (phosphorus will bind to iron or particles in sediments). The importance of internal loading during summer stratification is thus not well understood, but some potential pathways have been identified: the release of nutrients from sediments which have contact with epilimnion (i.e. shallow areas), turbulent flux through thermocline (Kortmann et al. 1982, Kamarainen et al. 2009), changes in the thickness and depth of metalimnion (Kortmann et al. 1982) and phytoplankton emerging from resting stages (Petterson et al. 1993). Whether, for example, turbulence leads to a flux of nutrients from hypolimnion to epilimnion might depend on the amount of iron in a lake: James et al. (1990) found that heavy turbulence in an iron-rich reservoir caused the re-oxygenation of iron and led to increased co-precipitation and sedimentation of iron and phosphorus. Consequently, no increases in epilimnetic phosphorus concentrations were observed. In contrast, Sorrano et al. (1997) found that during the summer in Lake Mendota, high turbulence did not increase the sedimentation rate and that the internal input of phosphorus from hypolimnion to epilimnion was ten times higher than external phosphorus inputs. It seems, therefore, that internal loading of nutrients can be an important factor in deep lakes also during summer months. In fact, multiple studies have estimated the scale of internal loading of nutrients in deep lakes (Nürnberg 1984, Larsen et al. 1981, Effler et al. 1986, James et al. 1990, Sorrano et al. 1997). The estimates vary depending on local conditions from 0% up to almost 80% of total nutrient loads to a lake ecosystem. However, still not enough is known about factors influencing the rate of nutrient flux from hypolimnion to epilimnion or about the different potential pathways.

Freshwater ecosystems are just a small percentage of the whole global surface area, but their global significance in nutrient and carbon cycles is high. For example, inland waters receive twice as much terrestrial carbon (Cole et al. 2007) and can bury four times more organic carbon than oceans (Downing et al. 2008). There is often an excess of carbon relative to nitrogen and phosphorus concentrations (which usually limit productivity) in freshwater ecosystems (Hessen and Anderson 2008), but carbon limitation of secondary production can still be an issue, at least in oligotrophic environments (Eiler et al. 2003). Generally, it seems obvious that the lack of a full understanding of both nutrient and carbon cycles within freshwater ecosystems and their changes during eutrophication or oligotrophication can be serious sources of error for both local and global nutrient and carbon budgets as well as for the management of these ecosystems.

Eutrophication and nutrient limitation

While various nutrients were already linked with ecosystem's productivity at the beginning of the 20th century (Weber 1907, Johnstone 1908), amounts of nutrients were not linked with the anthropogenic eutrophication problem until the 1960s. During the 1960s and 1970s, lots of effort was put into determining which of the main nutrients (carbon, nitrogen or phosphorus) is more important in limiting the productivity of freshwater ecosystems. Finding an answer to this question was of paramount importance, because it sets rules for the design of wastewater treatments in order to limit eutrophication, among other concerns. Thanks to studies performed by Vollenweider and Schindler at the Canadian Experimental Lakes Area (reviewed in Schindler 2006) it became established early on as a paradigm that phosphorus is the main nutrient limiting productivity in freshwaters (Vollenweider 1968, Schindler 1974, 1977) and that phytoplankton production and biomass can be explained by phosphorus loading after correcting for water renewal time (Schindler 1978). Vollenweider (1968) was also the first author to link changes in watersheds to the problems

in lakes. Vollenweider's report was never published in a peer-reviewed journal, but still became one of the most important pieces of evidence supporting phosphorus-induced eutrophication.

Another well-known example of phosphorus limitation comes from lake Washington. Before 1968 it received wastewaters which were only biologically treated, which caused deterioration of the lake's water quality. Edmondson (1970) determined that the eutrophication was caused by phosphorus based on a strong positive correlation between phosphate and chlorophyll *a* (chl *a*) concentrations. Following diversion of the sewage waters away from lake Washington, both chl *a* and phosphate concentrations began to decrease. However, a similar improvement was not observed for nitrate concentration (Edmondson and Lehman 1981), which indicated that phytoplankton biomass was indeed limited by phosphorus.

Despite these strong results showing the phosphorus limitation of phytoplankton growth, research into nutrient limitation still actively continues and various conclusions have been drawn. Recent publications about lake ecosystems indicate: limitation by phosphorus (Jeppesen et al. 2005, Schindler et al. 2008, Paterson et al. 2011), limitation by nitrogen (Vitousek and Howarth 1991) and equal limitation by nitrogen and phosphorus (Elser et al. 2007, Sterner 2008). Clearly, there must be more to the nutrient limitation than would appear at first glance.

The main argument which challenges nitrogen limitation has been that cyanobacteria are capable of fixing atmospheric nitrogen and thus are possibly supplying lake ecosystems with enough nitrogen to cancel out the effect of nitrogen limitation on the biomass of primary producers (Schindler et al. 2008, Vrede et al. 2009). Rather than influencing the biomass, nitrogen limitation might change the phytoplankton community composition in favour of cyanobacteria which are capable of fixing atmospheric nitrogen. At the same time, total biomass of primary producers would remain in proportion to phosphorus concentration. One of the main proponents of this idea is Schindler, whose experiments at the Experimental Lakes Area in Canada offer strong results in support of this (Schindler et al. 2008). However, when Scott and McCarthy (2010) performed an

independent analysis of the same data set, they concluded that phytoplankton biomass did indeed decrease when nitrogen limitation became stronger and that cyanobacteria were not capable of supplying enough nitrogen to the ecosystem to fully negate the nitrogen limitation.

The question of whether cyanobacteria can offset nitrogen limitation or not is vital for watershed management. If cyanobacteria do supply enough nitrogen in freshwater ecosystems, then there is no need to address nitrogen inputs in wastewater treatment. The treatment of nitrogen can even be counterproductive if the high costs associated with nitrogen removal lead to unwillingness to invest in wastewater treatment (Schindler and Hecky 2009). However, eventually discharged nitrogen will end up in marine and estuarine ecosystems, where nitrogen limitation is widespread (Blomqvist et al. 2004, Howarth and Marino 2006). Multiple factors, including a lack of micronutrients which are needed to support nitrogen fixation, cause nitrogen fixation to occur at much lower rates in marine systems than in freshwaters (Paerl 2009). Consequently, cyanobacteria are not able to offset nitrogen limitation in marine environments and high nitrogen inputs can lead to eutrophication. Thus, nitrogen cannot be ignored in wastewater management, even though its influence in freshwaters is questionable and may be limited to some lakes in the tropics (Hecky and Kilham 1988) and in New Zealand (Abell et al. 2010). Generally, in temperate lakes, the impact may be restricted to the promotion of cyanobacteria, which in itself can be an undesirable effect (Downing et al. 2001, Conley et al. 2009).

The ideas around how a "non-limiting" nutrient can influence community composition or how co-limitation by multiple nutrients can be common phenomena are elucidated under the concept of ecological stoichiometry (Sterner and Elser 2002, Elser et al. 2007, Saito et al. 2008). This concept is based on the principle that not only quantity but also the quality of food can limit the growth rate of an organism in a species-specific manner. Each phytoplankton species responds differently to nutrient enrichments, which is also the reasoning behind the common idea that a low supply of nitrogen tends to promote nitrogen fixing cyanobacteria in freshwaters (Downing et al.

2001, Vrede et al. 2009). Ecological stoichiometry emphasises that phytoplankton community composition and trophic structure, among other factors, are easier to understand when looking at nutrient balance in ecosystems as a whole and not only as either phosphorus or nitrogen limitation. "Nutrient limitation" should not be thought of as in the common textbook example of a leaking bucket (i.e. the nutrient which is in smallest supply in comparison to demand will determine the biomass in the ecosystem) but as a complex network of interactions and co-effects that determine not only biomass but also the composition of the community and functioning of an ecosystem.

It is possible that the management of freshwaters should be through the manipulation of the ratio between total nitrogen and total phosphorus (TN:TP ratio) rather than decreasing phosphorus inputs. For example, in the Canadian Experimental Lakes Area, lakes which were fertilised with both nitrogen and phosphorus maintained the same primary producer composition (only abundance increased) as unfertilised lakes (Kling and Holmgren 1972, Schindler 1998). In contrast, lakes fertilised with only phosphorus developed a high biomass of cyanobacteria.

Nutrient limitation of primary production can be estimated based on short- and long-term intervals. In the long-term, the limiting nutrient will limit the biomass that an ecosystem can support and possibly modify the structure of the community. In the short-term, the limiting nutrient can be evaluated based on the activity of organisms: for example, which enzymes associated with nutrient acquisition are most active or which nutrients give the biggest response in growth rate in a nutrient addition experiment. Generally, over long time scales, phosphorus is ultimately the nutrient-limiting biomass in an ecosystem, but at shorter time scales co-limitation with other nutrients is expected (Sterner 2008). The TN:TP ratio is one of the most well-known and most used indicators of nutrient limitation (for example Klausmeier et al. 2004, Schindler et al. 2008, Vrede et al. 2009). Redfield's ratio of 16 nitrogen atoms to one phosphorus atom (about 7:1 as weight ratio) is commonly used as a general set point determining whether nitrogen or phosphorus is the limiting nutrient of

phytoplankton growth, although an optimal ratio seems to be species-specific (Klausmeier et al. 2004) .

All factors which can cause nutrient balance changes in an ecosystem can potentially also influence the TN:TP ratio. Eutrophication is generally induced by high phosphorus inputs, and consequently eutrophic status correlates with low TN:TP ratio; in contrast, oligotrophic status correlates with high TN:TP (Downing and McCauley 1992, Jeppesen et al. 2005). Such factors can cause changes in the nutrient limitation of an ecosystem with time and is therefore a dynamic characteristic of an ecosystem.

The structure of the primary producer community is not only dependent on nutrient balance: consumers strongly modify the producer communities and, generally, strong changes in the structure and composition of food webs are expected during eutrophication (Carpenter et al. 1995).

Oligotrophication - regulators and effects

Oligotrophication can be defined as a decrease in the rate of supply of organic matter to an ecosystem (Nixon 2009). In the context of this thesis and as is often common in the literature, the term oligotrophication is used in its more narrow meaning, specifically signifying the recovery of a lake ecosystem from a eutrophic state which was originally caused by high anthropogenic nutrient inputs (Schindler 2012). Earlier terms used in the literature were re-oligotrophication (for example Jeppesen et al. 2005) and sometimes de-eutrophication (for example Niemelä et al. 2002).

In regards of freshwater ecosystems, it has generally been accepted that reducing phosphorus inputs is the best way to achieve oligotrophication. Implementation of the reduction has led to an improvement in the ecological state of many lakes (summed up in Anderson et al. 2005). However, the recovery process can be long, commonly taking 10-15 years (Jeppesen et al. 2005, Søndergaard et al. 2005). The reason for this is often speculated to be the internal storage and release of phosphorus, which remains in the system much longer than nitrogen. Reductions in

nitrogen concentrations are commonly achieved within 0-5 years (Jeppesen et al. 2005). In contrast, during eutrophication it can take 10-20 years until nitrogen concentrations reach a steady state (due to the short period of time each year available for nitrogen fixation; Schindler et al. 1987, Schindler 1998).

Despite the difference in the reaction rates of nitrogen and phosphorus to input reductions, the fact remains that the reductions often target phosphorus. This could be one of the reasons why the TN:TP ratio often increases during oligotrophication (Anderson et al. 2005, Jeppesen et al. 2005) instead of decreasing, as would be expected based on the reaction rates of nitrogen and phosphorus concentrations. This relationship (higher TN:TP in lakes with low TP) was previously found by, for example, Downing and McCauley (1992), when comparing lakes from a wide spectrum of trophic states. This indicates that high TN:TP ratios generally occur in oligotrophic and mesotrophic lakes and that the changes observed by Jeppesen et al. (2005) were not only related to lakes going through oligotrophication. Overall, it seems that changes in nutrient limitation of primary production during oligotrophication are not yet fully understood.

Delays in an ecosystem's response to oligotrophication also occur with the reaction of phytoplankton: Dokulil and Teubner (2005) found that the highest phytoplankton biomass was reached after reductions to nutrient inputs had already been implemented and that a decline in biomass occurred for the first time after six years had passed from the implementation. Also, changes in the phytoplankton's community structure did not follow the pattern which occurred during eutrophication, but was more complex and less predictable.

Currently, there is not enough information about oligotrophication to predict in detail the responses of an ecosystem during its occurrence. This can seriously hamper the management of the ecosystem and makes it impossible to estimate risks to the ecosystem caused by other simultaneous stressors, for example climate change and decreasing biodiversity.

A somewhat better known side of oligotrophication is ecosystem sensitivity towards external inputs. The responsiveness of lakes towards changes in external nutrient loading can be divided to three groups: reversible (recovery is immediate and directly proportionate to phosphorus input reductions), hysteretic (recovery needs extreme reductions in inputs over prolonged period of time) or irreversible eutrophication (recovery cannot be achieved by input reductions only, Carpenter et al. 1999). The irreversible eutrophic status is caused by high hysteresis of the ecosystem, which occurs when internal features and processes of the ecosystem maintain the eutrophic status. The main regulators behind the hysteresis are water retention and the internal cycling of phosphorus. In an ecosystem which has high hysteresis, reductions of external inputs will not have much of an effect and other restoration measures are needed.

The effect of water retention time on a lake's sensitivity towards reduced nutrient load is twofold: a very fast renewal rate can be the main factor regulating phytoplankton biomass which means that input reductions do not matter. In contrast, in deep and large lakes, a long retention time and large volume can slow the effect which the reduction of external nutrient inputs has on the total nutrient concentrations within lake and thus delay the lake's response (Reynolds 2003). The second main factor which can inhibit oligotrophication, even of stratified lakes, is internal loading of nutrients from sediments (Phillips et al. 2005, Mehner et al. 2008).

The significance of internal loading as a factor inhibiting oligotrophication is well established with regards to shallow lakes, but it is still not a well-known factor in deep lakes and its importance there should be thoroughly estimated. Other important parameters for the estimation of a lake’s sensitivity towards changes in external inputs are, for example: (i) limnological characteristics (lake retention time, existence of inlets and outlets, ratio between shoreline length and area of lake, and percentage of shallow areas (Reynolds 2003, Bajkiewicz-Grabowska 2011), the ratio between a sum of basin and catchment areas to lake volume i.e. Schindler's ratio,

(Schindler 1971)), (ii) local weather (climate zone, strength and direction of winds, precipitation) and (iii) features of the drainage area (land use and degree of anthropogenic pressure).

In general, water management programs such as the EU's Water Framework Directive assume a linear and constant response of aquatic ecosystems to reductions in nutrient inputs; therefore, a historical reference status of a particular lake could be obtainable through a combination of nutrient input reductions and possibly through other management practices. However, trying to obtain some previous state of a lake might be very unrealistic (Duarte et al. 2009). Multiple factors other than phosphorus (carbon dioxide concentration, climate forcing, fish stocks, water level, habitat and species richness, etc.) also influence chl *a* concentrations and their base values often change with time (Duarte et al. 2009). Thus, especially when climate change is becoming a stronger determining factor for the ecological state of lakes, it would be a more profitable use of resources to concentrate management efforts into improving or maintaining ecosystem functions rather than trying to obtain target nutrient values.

Other factors influencing a lake's trophic state

As has been described, external nutrient inputs, internal nutrient cycles and factors which modify these are the main reasons behind a lake's trophic state. However, many other factors can also influence the trophic state of a lake ecosystem and their effect can be quite large under certain environmental conditions. One of the best known examples is the top-down control of phytoplankton biomass, where the high biomass of piscivores causes strong grazing pressure on phytoplankton, leading to lower phytoplankton biomass and chl *a* concentrations. Top-down control might be a generally more important mechanism in shallow lakes than in deep lakes (Jeppesen et al. 1997). In deep lakes, light conditions and nutrient limitation of phytoplankton growth influence the strength of top-down control (Benndorf et al. 2002).

Another important factor is climate change, which can strongly influence the ecology of lakes (Shimoda et al. 2011). Multiple factors which can influence the trophic state and functioning of lake ecosystems are regulated by temperature. The stratification of deep lakes causes a barrier for the mixing of water and possibly for internal loading. As the stratification is strongly regulated by temperature, climate change might be able to influence many properties of lakes (for example depth of epilimnion, light environment for phytoplankton and onset of stratification). Also, other factors, such as the timing of ice formation and thaw (Magnuson et al. 2000), external nutrient inputs (Jeppesen et al. 2009, 2011) and individual metabolism (Woodward et al. 2010), can all be influenced by climate. A significant aspect of metabolism changes is that warming weather can increase the rate of respiration faster than the rate of primary production, which can lead to a reduction in the lake's carbon sequestration (Yvon-Durocher et al. 2010). Generally, the effects of climate change in lakes are expected to be similar to eutrophication and cyanobacteria are expected to benefit from many of them (Elliott 2012). When so many factors are changing, it can be safely speculated that the functioning of lakes and their effect on global carbon and other nutrient cycles will be significantly different in the future.

For freshwater areas, it is characteristic to collect and incorporate inputs from the surrounding landscape and thus reflect changes occurring in the environment. It is therefore not surprising that lakes and reservoirs have been called sentinels of change: they give the first indications of things going wrong or improving (Adrian et al. 2009, Williamson et al. 2009). The reasons behind this sensitivity are multiple: lakes are often fragmented habitats with limited possibilities of dispersal by a species, they are already stressed by anthropogenic inputs, and water temperature and availability are dependent on weather (Woodward et al. 2010). Already, temperature increases in many temperate lakes have been higher than in their surrounding terrestrial areas (Schneider and Hook 2010). Consequently, lakes and reservoirs will be one of the first ecosystems to show any serious effects of climate change.

The effect of climate change on hypoxia is not clear. Even though an increase in temperature decreases oxygen saturation of water, other factors such as changes in wind levels, the occurrence of storms and the trophic state might be more important. However, under specific unfortunate circumstances, an increase in hypoxia might become an issue. In aquatic ecosystems, hypoxia can cause changes in the internal cycling of nutrients and can also lead to fish deaths or other undesirable outcomes (Conley et al. 2009). Once hypoxia occurs, its reoccurrence becomes more likely and recovery can take up to a decade (Steckbauer et al. 2011).

In addition to increased temperature, extreme events are also likely to become more common in the future (Barriobedro et al. 2011, Rahmstorf and Coumou 2011), which implies that impacts caused by climate change on an ecosystem will not occur gradually but rather in strong and patchy changes (Breshears et al. 2011). At the most extreme, this could lead to fast crashes of ecosystem services (for example when drought destroys a forest or a wetland area). All of these effects in the functioning of ecosystem and other frequent disturbances can weaken an ecosystem's resilience and cause changes from which it can be difficult to recover.

Investigations into how climate change influences ecosystems have three basic methods: tracking effects *in situ*, usage of models and also utilisation of years with unusual weather patterns for estimation of the impact of climate forcing on ecological variables. The last option is a rough method, where it can be difficult to separate multiple weather-related components from each other and to conclude that something was caused by factors related to climate change rather than some other simultaneously changing variable (for example changes in the catchment area). Nevertheless, the method has been used previously and has yielded some interesting and potentially important results (for example Wolford and Bales 1994, Nowlin et al. 2004, Nõges et al. 2011). For example, Nõges et al. (2011) found that the effects of increased precipitation and nutrient input to lakes were higher in a lake with a low trophic state than in a lake with a high trophic state.

Opportunities for empirical short-term studies are likely to rise in the future, because the likelihood of extreme events is expected to increase with climate change (Rahmstorf and Coumou 2011). These can be seen as scientific opportunities for investigating phenomena which will probably become more common in the future. Observing them is important in order to be able to recognise all potential impacts which extreme events and gradual climate change can bring about, and to take the effects into account when planning management strategies for lakes or catchment areas.

Generally, expected changes caused by climate change in Central Europe are: a high rise in peak summer temperatures, precipitation increase during winter and decrease during summer, higher likelihood of summer drought and shorter snow season (Christensen et al. 2007). Weather in Central Europe is strongly influenced by large scale climatic and oceanic phenomena such as North Atlantic Oscillation and the Gulf Stream; there is uncertainty over how climate change will influence these forces. It seems that the ocean circulations might weaken, but that this would not lead to cooling in Europe, as was previously expected (Christensen et al. 2007). It is important to remember that unusual weather conditions do not have to be related to human-caused climate change, but they can be a result of natural variance in large scale meteorological patterns; also, the effects of climate change vary a lot according to region, even causing contrasting effects in different parts of the globe.

It is nowadays common that multiple strong but relatively weakly understood trends of change (mainly oligotrophication and climate change) are occurring in many lakes at the same time. Thus, it is important to identify how these trends can influence each other and whether there are going to be any potential problems. Lakes are a sensitive and ecologically and economically important environment where relatively small changes can lead to big outcomes. Nobody has previously taken a comprehensive look at the present state of the GML-system and its potential state in the future. Information about past and present trophic states and nutrient balance are

essential for understanding its current ecological state, its future and also problems which might cause undesirable outcomes in the system. All management decisions should be based on proper information about their ecological risks and outcomes, but such knowledge has not been available for the GML-system.

Goals of the study

The aim of this research was to evaluate the ecological state of the GML-system during recent decades (mainly from 1990 onwards), with focus on water quality and biology. Priority was placed on identifying which internal and external factors influenced the past and present ecology of the GML-system and whether they changed with time. Based on these results, the goal was to estimate how the possible changes affected the ecosystem. Factors which caused or influenced the present ecological state of the GML-system were to be identified. A forecast of the potential ecological future of the system was to be formed. An additional goal was to evaluate any possible problems related to management of the system and how they could be resolved in the future.

MATERIALS AND METHODS

Study area

The study was conducted at a pelagial zone of 17 lakes of the GML-system, which are situated in the North-Eastern part of Poland (fig 1). The area was shaped by ice age and has moraine ground. The lakes are connected naturally or through artificial canals. Land use in the drainage area of the GML-system includes agriculture, towns, dispersed settlements and forests. The lakes are used for fishery, recreational purposes and tourism.

The GML-system is divided to two different areas: the northern part belongs to the Pregoła catchment and the southern to the Pisa catchment. According to Bajkiewicz-Grabowska (2008), the location of drainage divide may have at some point changed. Despite the division to two different catchments, the GML-system is characterised by similar geological characteristics of the lakes.

Information about the basic morphological characteristics of the studied lakes is shown in table 1. Data were collected from Waluga and Chmielewski (1998, 1999) and Jańczak (1999). Most of the lakes are dimictic deep lakes, which have a clear stratification structure during summer months. Lakes Szymon and Śniardwy are the only clearly polymictic lakes, and Lake Szymon the only shallow lake. Lakes Łabap, Niegocin and Boczne have characteristics from dimictic and polymictic lakes, especially at the chosen sampling sites.

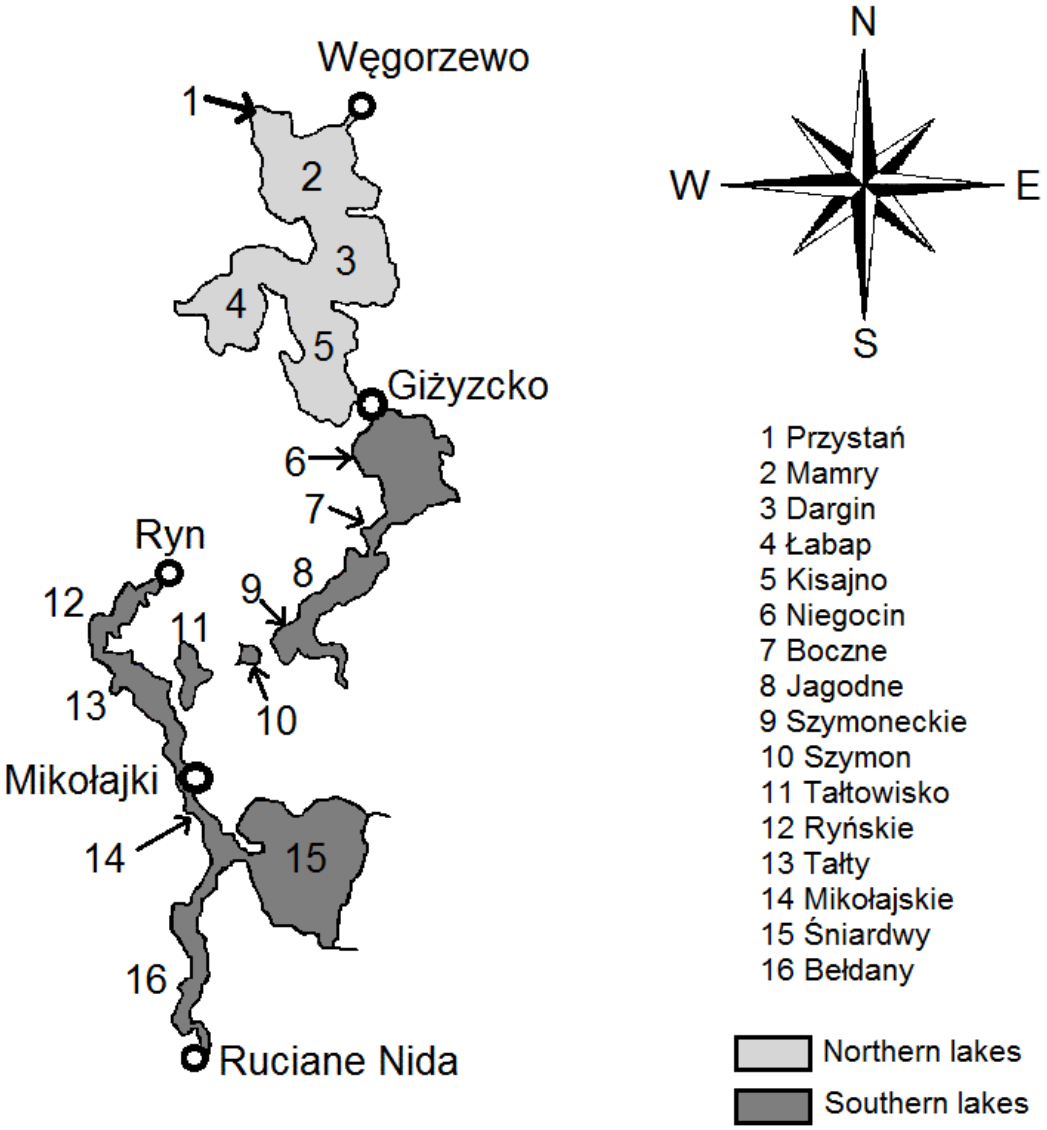

Fig 1. Map of the research area.

Table 1. Abbreviations, morphometric characteristics and trophic state index of sampled lakes (Waluga and Chmielewski 1998, 1999, Jańczak 1999). The first five lakes listed are part of the northern catchment, while the rest belong to the southern part.

Lake	Abbreviations	Area [ha]	Max depth [m]	Mean depth [m]	Depth at sampling site [m]	Direct drainage area [km^2]	Length of shoreline [m]	Mixing type
Przystań	Prz	115	45.6	13.4	31.2		34000	dimictic
Mamry	Mam	2 004	47.0	11.7	18.5	31.3		dimictic
Dargin	Dar	2680	37.6	10.6	23.4	87.1	32800	dimictic
Łabap	Lab				13.3			poly/di
Kisajno	Kis	1896	25.0	8.4	16.1		50100	dimictic
Niegocin	Nie	2600	39.7	9.9	12.1	51.7	34000	poly/di
Boczne	Bocz	183	17.0	8.7	13.0		8150	poly/di
Jagodne	Jag	943	37.4	8.7	21.9	89.5	35410	dimictic
Szymoneckie	S-kie				26.9			dimictic
Szymon	Szy	154	2.9	1.1	2.0		5800	polymictic
Tałtowisko	T-sko	327	39.5	14.0	33.1	72.1	11500	dimictic
Ryńskie	Ryn	676	50.8	13.8	36.0	53.4	27700	dimictic
Tałty site 1	Tal1	1160	44.7	13.5	32.2		31000	dimictic
Tałty site 2	Tal2				22.8			
Mikołajskie	Mik	498	25.9	11.2	26.1	14.1	15100	dimictic
Śniardwy	Sni	11340	23.4	5.8	11.2	15.0	97150	polymictic
Bełdany	Bel	941	46.0	10.0	24.3	46.1	34400	dimictic

The samples were collected at the pelagic zone, at a fixed location at one of the deepest sites of each lake, and were used to describe ecological conditions in each of the lakes. An exception was Lake Tałty, which had two sample collection sites due to high differences in anthropogenic pressure at different locations. Site 1 was located in the northern half of the lake (represents less eutrophic conditions) and site 2 was located close to a water treatment plant's effluent discharge. Generally, samples representing both limnetic (determined as the euphotic area outside of littoral zone, depth calculated as twice Secchi depth) and profundal (determined as zone below limnetic zone) zones were collected from all lakes. An exception was Lake Szymon, which is a shallow lake without a clearly defined limnetic zone.

In situ measurements and the collection of samples

Monitoring of the GML-system was carried out during mid-summer when stratification was well developed and the clear water phase had passed (August 2009, July, August 2010 and July, August, September 2011), and also during the spring over-turn (April 2009 and May 2011).

At each sampling site, before taking samples, depth profiles of temperature, pH, oxygen concentration and saturation were measured *in situ* with the YSI 6600-meter (Yellow Spring Instruments, USA). Secchi disk visibility was determined by a commonly used standard method.

Samples representative for limnetic zones were obtained by combining vol/vol subsamples collected at one metre intervals from 0.5 m below the surface down to double Secchi disk visibility. Profundal samples were collected from a water layer one metre above sediments. All samples were collected in polyethylene containers which had been washed with phosphorus-free laboratory-grade detergent and flushed with sample water just before taking a sample. Samples were transported to the laboratory within three hours of their collection.

Determination of chemical parameters

For chemical analyses, non-filtered and filtered (through GF/F glass fibre filters, Whatman) samples were collected and stored at -28°C. All non-disposable plastic and glass equipment were carefully washed with phosphorus-free laboratory-grade detergent and (for the analysis of dissolved organic carbon, phosphorus and various nitrogen forms) with 10% HCl. Just before use, all bottles were flushed with sample water.

Nitrogen

Total Kjeldahl nitrogen was determined in unfiltered samples as ammonium (NH_4^+) by the Solorzano method (1969), and after mineralisation according to Koroleff (1983b). Nitrate

concentration was measured in filtered samples colorimetrically following the phenoldisulphonic acid method (Taras 1950). Total nitrogen (TN) was calculated as a sum of total Kjeldahl nitrogen and nitrate. Nitrite was not included to the TN estimation, because its concentrations in limnetic waters were low, within the range of statistical errors of mean TN concentrations.

Phosphorus

Total phosphorus (TP) concentrations were assayed as phosphate (PO_4^{3-}) after wet oxidation of unfiltered samples with potassium persulphate and sulphuric acid (Koroleff 1983a).

Sulphate and chloride

Sulphate and chloride were determined from unfiltered water samples by utilising colorimetric Spectroquant test kits by Merck (catalogue numbers 114548 and 114401, respectively).

Dissolved organic carbon

DOC concentrations in filtered water samples were analysed with a Shimadzu TOC 5050 carbon analyser.

Oxygen concentration (for respiration rate assay)

Oxygen concentrations were measured according to the classical Winkler's method (recommendations for procedure in Carpenter 1965).

Determination of biological variables

Phytoplankton and number of heterocysts

Samples (300 mL) for phytoplankton and heterocyst analyses were fixed with Lugol's solution (added until an amber colour was reached) and supplemented with formaldehyde (5 mL). Samples were stored in cold and dark conditions. The number of algal and cyanobacterial cells, composition of phytoplankton as well as the quantity of cyanobacterial heterocysts in fixed water samples were counted under a light microscope Olympus CX-21 using Sedgwig-Rafter's chamber

(0.55 mL) according to the method by Wetzel and Likens (1991). The number of phytoplankton cells was expressed as cells mL^{-1}, treating structural units typical for a given species (single cell, colony or trichome) as one individual. Phytoplankton biomass was estimated based on a cell's or individual's biovolume assuming that 10^6 μm^3 equals 1 μg of fresh biomass (Kawecka and Eloranta 1994). Biovolume was calculated from the mean cell size obtained from microscopic measurements with an approximation of cell shapes to simple geometrical solids, which were closest to a given microorganism (Wetzel and Likens 1991, Kawecka and Eloranta 1994, Hutorowicz 2005).

Chlorophyll a

Phytoplankton cells were collected on glass fibre filters (GF/F Whatman) and chlorophyll *a* (chl *a*) was extracted with 98% acetone. The concentration was measured fluorometrically (TD-700 Turner Biosystems, USA) following a procedure by Arar and Collins (1992).

Enzymatic activity

Maximal, potential (Vmax) activities of L-leucine-aminopeptidase (AMP) and alkaline phosphatase (APA) were determined using the method of Chróst and Siuda (2006) as an increase in fluorescence when non-fluorescent substrates were hydrolysed to highly fluorescent products. Stock solutions of L-leucine-methyl-cumarinylamide hydrochloride (LAMC) and 4-methylumbelliferyl phosphate (MUFP) were prepared to a concentration of 2 mM in 96% ethanol and in dimethyl sulphoxide, respectively, and stored at -28°C. The stock substrate solutions were diluted with appropriate solvents to 0.01, 0.02, 0.04, 0.1, 0.2, 0.4, 0.6 and 0.8 mM immediately prior to assay.

For both enzyme assays, 0.1 mL of respective substrate solutions were added to 3.9 mL of sample water, yielding final concentrations of 0.25, 0.5, 1.0, 2.5, 5, 10, 15 and 20 μM. Subsamples were incubated in the dark for 0.5-2 hrs, depending on the expected enzyme activity. Fluorescence (excitation 380, emission 440 for AMP and excitation 365, emission 460 for APA) was measured in a Shimadzu RF 1501 spectrofluorometer at T_0 time and after incubation.

The enzymatic reactions followed Michaelis-Menten kinetics. The data were analysed with

the direct plot method as described in Chróst and Siuda (2006) using Origin (Origin LabCorporation) software.

Respiration rate

Planktonic respiration rate was measured as the rate of oxygen consumption in the dark in unfiltered water samples based on the classical dark bottle method (Carpenter 1965, Pace and Prairie 2005) and a modified method (Kauppinen et al. 2013). A series of black coloured 110-120 ml Winkler's bottles (25–30) were filled with limnetic sample water and incubated in an incubator for 72-96 hours. The temperature of the incubator was equal to the mean temperature of the environment (epilimnion) that the samples were taken from. At time T_0 and after each 8–10 hours of incubation, triplicate samples were treated with reagents and oxygen concentrations were measured. A decrease in O_2 concentration during the incubation period was recalculated into a change in C-CO_2 concentration.

For the classical dark bottle method, respiration rate (Rr) was determined as the rate of change in C-CO_2 concentration between T_0 and T_1 (a maximum of 8-10 hours of incubation). For the modified method, the respiration rate was calculated assuming that the increase in C-CO_2 concentrations during the incubation can be described by equations: $f(t) = Rr \times t$ for the linear relationship and $f(t) = (OM_{Resp} \times t) / (k + t)$ for the hyperbolic relationship. Wherein: Rr = respiration rate, t = duration of incubation, OM_{resp} = total respirable organic matter pool present in lake water, and k = model coefficient. In order to determine respiration rate, the functions described above were differentiated. For linear function, the value of f'(t) was constant and equal to the slope coefficient (Rr). For hyperbolic function, the changes in C-CO_2 release rate during incubation were described by the function of first derivatives: $f'(t) = dy/dt = (OM_{Resp} \times k) / (t + k)^2$. This allowed respiration rate at incubation time t=0 to be calculated and thus, the initial respiration rate (Rr) in the environment to be estimated.

Primary production and extracellular release

To determine planktonic primary production, the classical light-dark bottle method (Howarth and Michaels 2000) and C^{14} method (Chróst and Siuda 2006) were used simultaneously. In the case of both methods, samples were incubated in a luminostate at *in situ* temperature (equal to mean temperature of epilimnetic layer at sampling time) in cool white illumination at16 µmol photons m^{-2} s^{-1} provided by fluorescent tubes. Extracellular release was determined by the C^{14} method as described by Chróst and Siuda (2006).

The percentage of extracellular release (PER) was calculated based on the net primary production rate (NPPr). Gross primary production rate (GPPr) was determined as a sum of NPPr and Rr for both the light-dark bottle and C^{14} methods. Specifically, NPPr obtained from the light-dark bottle method was summed up with Rr obtained from the dark bottle method. NPPr obtained from the C^{14} method was combined with Rr results obtained from the modified method.

Determination of physical variables

Variables describing the stratified structure of each lake (except the shallow lakes Śniardwy, Szymon and Łabap which do not have a stratified structure) were determined using data collected *in situ*. Epilimnion was defined as the zone where the temperature change was less than 1°C per metre depth (i.e. the top layer of a thermally stratified lake). Mean temperature and pH of the epilimnion were counted as a mean of the highest and lowest values encountered in the epilimnetic layer. The ratio between depths of euphotic (i.e. limnetic) zone and epilimnion was used as a measure of phytoplankton light limitation.

Hypoxia was defined as a condition when the oxygen saturation of water was less than 25%. The upper border of the hypoxic water layer was estimated from depth profiles assessed by an oxygen probe of YSI 6600-meter. The thickness of the hypoxic water column was calculated as the distance between the maximum depth at the sampling site (table 1) and the upper border of the

hypoxic water layer. The distance between the lowest part of epilimnion and hypoxic zone was calculated and used to describe the thickness of an oxygenated buffer zone (OBZ). This zone corresponds to an oxycline from about 100% to 25% oxygen saturation.

Vertical entrainment was calculated according to Blanton (1973) based on temperature profile data collected with YSI 6600-meter. Blanton's formula for entrainment was: $(H_2-H_1)/(T_2-T_1)$, where the depth, H was located at a constant temperature that remained within the thermocline throughout the time period (T_2-T_1).

External datasets

Historical database

Historical ecological data concerning the GML-system were collected from published literature (full reference list available from Siuda et al. 2013) and from previously unpublished results of the Microbial Ecology Department at the University of Warsaw. This database spanned a time period from 1960 to 2008. However, only results from spring (April-May) and summer (June-September) seasons and on variables phosphorus (TP), Secchi disk visibility and chl *a* concentrations were taken into account.

External nutrient inputs

Information about external inputs was collected from various online databases. Data about usage of mineral fertilisers in Poland was obtained from the IFA (International Fertilizer Industry Association). Regional data about sewage treatment, mineral fertiliser usage and agricultural area were obtained from Local Data Bank (Bank Danych Lokalnych, BDL 2012) and from yearly reports by the Statistical Office in Suwalskie (SOS 1981-1996). SOS and BDL are part of a state owned and organised statistical office which collects and publishes official statistical information. The reported amounts of discharged pollutants from wastewater treatment plants by the statistical office are based on yearly or half-yearly reports which are provided by the authorities of the

treatment plants. The regional data about the consumption of mineral fertilisers provided by the statistical office were elaborated based on data collected from production and trade units, statistical reports of agricultural farms and expert's estimates (GUSDR 2012).

Characteristics of drainage area

Information about lake area, depth, volume and drainage area were collected from available literature (Waluga and Chmielewski 1998, 1999, Jańczak 1999). Land use in the direct surroundings of lakes and the presence of vegetative riparian buffers were determined from available maps (mainly satellite photos from Google Maps).

Analyses based on external datasets

External nutrient inputs

Point nutrient loads were estimated based on data available from BDL (2012). These values do not include industry or other potential point sources of nutrients.

Characteristics of drainage area

The influence of drainage area characteristics on the trophic state of a lake is estimated through a type of analysis presented by Reynolds 2003 and Bajkiewicz-Grabowska 2011 for example. Variables to characterise the direct drainage area and lake basin were chosen based on available data that showed differences between lakes. These variables were categorised and points for each lake were appointed based on the category.

Statistics

The software packages Statistica 10 and Origin 8.6 were used for all statistical analyses. Welch's t-test (i.e. unequal variances t-test) was used to compare means between groups of data. In a few cases, the Kruskal-Wallis test was used instead. Confirmations of normal distributions were

made with Chi-square test.

Long term changes in trophic state

TSI changes with time were analysed by combining two datasets together (data from the database for 1980 to 2008 and from the present investigations for 2009-2011).

In order to compare changes in TP and chl *a* concentrations, the values were standardised [z = (x-mean)/SD]. A time series analysis was performed on the standard scores. Missing values were interpolated from adjacent points.

Influence of nutrient limitation on cyanobacterial assemblages

Ecological factors which promoted growth rates of cyanobacteria were estimated with a forward stepwise multiple regression. All routinely measured variables were included in the analysis. Default F-values to enter and remove variables from a regression model were used (F-value to enter = 1, F-value to remove = 0.1). A principal components analysis was performed on all variables in order to confirm selection of the entered variables by the forward stepwise multiple regression. Before analysis, distribution of the entered variables was determined. All showed log-normal or Poisson-distributions; thus, $\log_{10}$-transformations were performed on all of them. A new multiple regression was performed on these selected and transformed variables using all effects. Predicted results and residuals were counted after removal of the log-transformation. Goodness of the obtained model was evaluated by analysis of residuals. The analysis included evaluation of the presence of outliers and of trends in the variation of residuals. Also, the normal distribution of residuals was confirmed.

RESULTS

Coverage of routinely measured data (TP, TN, chl *a* concentrations and Secchi disk visibility) is presented in table 2. In September 2011, only TP and TN concentrations were analysed. All of the following results concern the limnetic zone, unless otherwise stated.

Table 2. Routine samples collected during 2009-2011. Explanations for signs: +/+ euphotic zone and profundal samples collected, + only euphotic zone sample collected, * oxygen and temperature profile data.

	04-2009	08-2009	07-2010	08-2010	05-2011	07-2011	08-2011	09-2011
Przystań		+/+ *	+/+ *	+/+ *	+	+ *	+	+
Mamry		+/+ *	+/+ *	+/+ *	+	+ *	+	+
Dargin		+/+ *	+/+ *	+/+ *	+	+ *	+	+
Łabap		+/+ *	+/+ *	+/+ *				
Kisajno		+/+ *	+/+ *	+/+ *	+	+ *	+	+
Niegocin	+	+/+	+/+ *	+/+ *	+	+ *	+	+
Boczne	+	+/+	+/+ *	+/+ *	+	+ *	+	
Jagodne	+	+/+	+/+	+/+ *	+	+ *	+	+
Szymoneckie	+	+/+	+/+ *	+/+ *		+ *	+	
Szymon	+		+	+	+	+	+	+
Tałtowisko	+	+/+ *	+/+ *	+/+ *	+	+ *	+ *	+
Ryńskie	+	+/+ *	+/+ *	+/+ *	+	+ *	+ *	
Tałty site 1	+	+/+ *	+/+ *	+/+ *	+	+ *	+ *	+
Tałty site 2	+	+/+ *	+/+ *	+/+ *		+ *		
Mikołajskie	+	+/+ *	+/+ *	+/+ *	+	+ *	+ *	+
Śniardwy	+	+/+ *	+/+ *	+/+ *	+	+ *	+ *	+
Bełdany	+	+/+ *	+/+ *	+/+ *	+	+ *	+ *	+

Long-term changes in trophic state of the GML-system

To evaluate changes in the trophic state of the GML-system during previous decades, results from historical database (1960-2008) and from the present investigations (2009-2011) were combined. Long-term changes in the trophic state of the southern and northern lakes were analysed separately (fig 2).

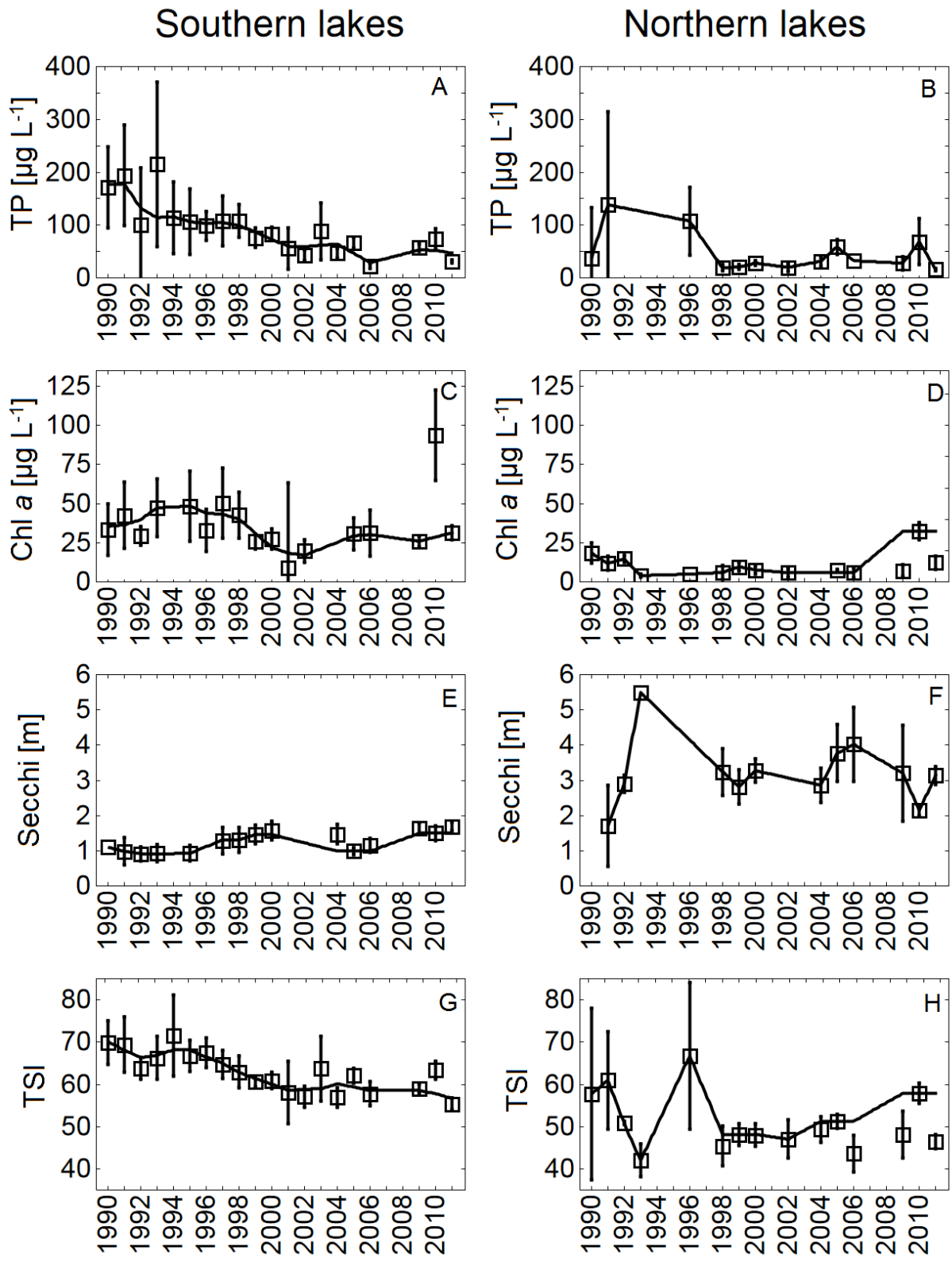

Fig 2. Time series of mean TP and chl *a* concentrations, Secchi disk visibility and mean TSI values in the southern (A, C, E, G) and the northern lakes (B, D, F, H) from 1990-2011. Data for 1990-2008 were obtained from the database of Microbial Ecology Department and data for 2009-2011 were obtained during the present investigations. Mean values or single data points are shown with squares and standard deviations with thin lines. Lowess curves were fitted on the data.

Intensive eutrophication began in the southern lakes in the 1960s and continued until the middle of the 1990s (fig 2 A, C, E and G). Generally, TP concentrations eventually decreased to about one fourth of the highest values found during the 1980s. Mean TP was 206 µg L^{-1} (SD 168) during the 1980s, 124 µg L^{-1} (SD 90) during the 1990s and 51 µg L^{-1} (SD 31) during 2000-2011 (fig 2 A). Chl *a* concentrations in the southern lakes were highly variable and there was no observable trend. Mean was 38 µg L^{-1} (SD 24) during the 1990s and 37 µg L^{-1} (SD 39) during 2000-2011 (fig 2 C). Secchi disk visibility improved from 0.9 metres (SD 0.3) in the 1990s to 1.5 (SD 0.5) metres during 2000-2011 (fig 2 E). Mean TSI was also high during the 1980s (72, SD 12). From approximately 1990 onwards, the trophic state began to decrease. Mean TSI was 67 (SD 8) during 1990-1994, 64 (SD 7) during 1995-1999 and 59 (SD 7) during 2000-2004 (fig 2 G). Oligotrophication appears to have stopped after 2004. Mean TSI remained at about the same level (59, SD 6) during 2005-2011. All of these variables (TP, Secchi disk visibility and chl *a*) showed sudden high changes in 2010. The changes which occurred in 2010 will be described in detail in a following chapter.

Long-term trends in trophic state of the northern lakes were not clear (fig 2 B, D, F and H). There was a low frequency of measurements, especially from the 1980s and early 1990s. Mean TP was 52 µg L^{-1} (SD 64) during the 1990s and 34 µg L^{-1} (SD 24) during 2000-2011 (fig 2 B). Neither chl *a* concentration nor Secchi disk visibility changed significantly with time; their means were 12 µg L^{-1} (SD 9) and 3.2 m (SD 1.8) during 1990-1999 and 12 µg L^{-1} (SD 10) and 3.2 m (SD 1.0) during 2000-2011, respectively (figs 2 D and F). A distinct trend was also not visible in mean TSI, which was 46 (SD 9) during the 1980s, 52 (SD 10) during the 1990s and 49 (SD 6) during 2000-2011 (fig 5 H). Concentrations of TP and chl *a*, as well as Secchi disk visibility, showed great changes in the northern lakes in 2010.

Trends in trophic state were more closely analysed in lakes Niegocin, Mikołajskie and Ryńskie (fig 3).

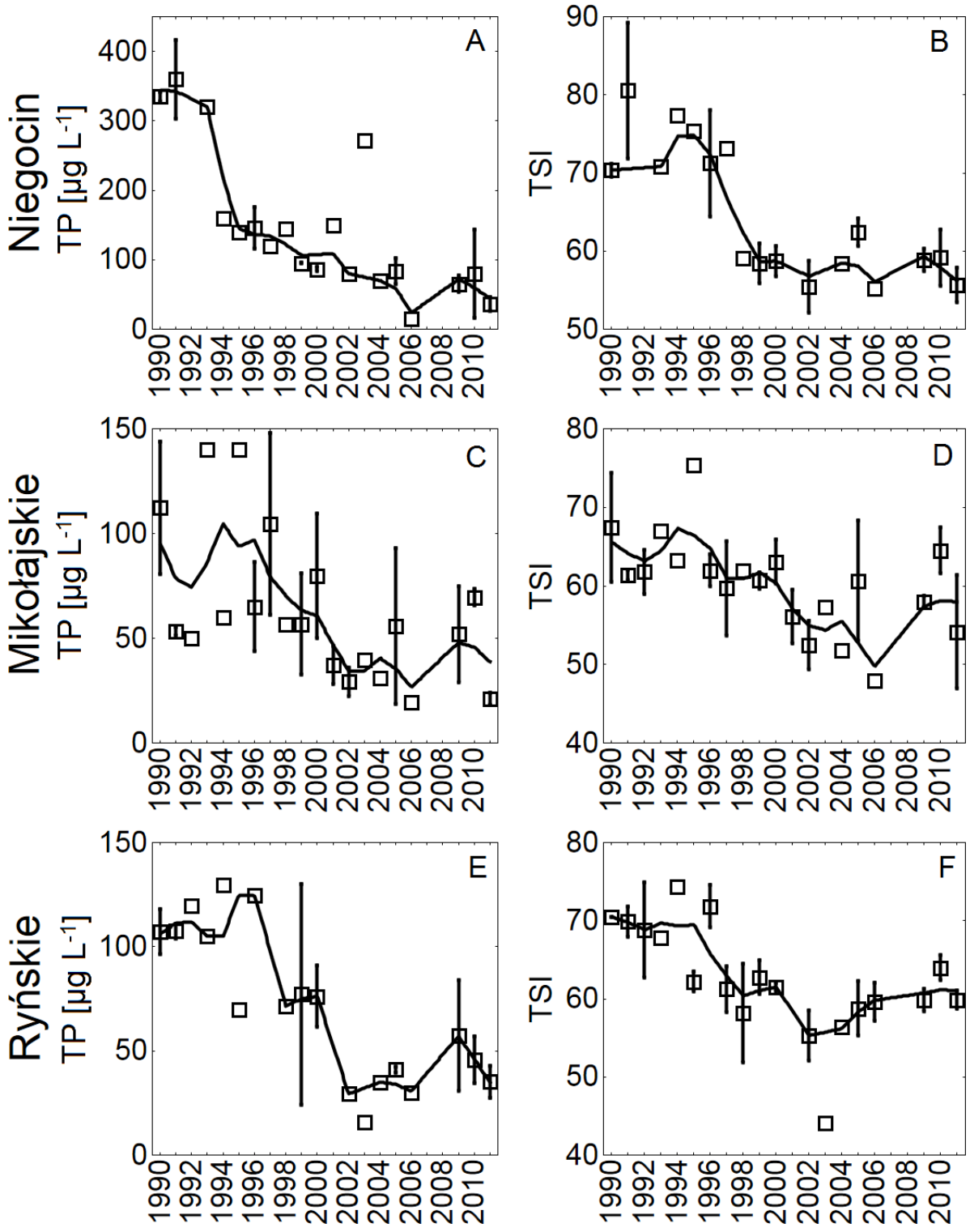

Fig 3. TP and TSI changes in lakes Niegocin (A, B), Mikołajskie (C, D) and Ryńskie (E, F). Data for 1990-2008 were obtained from the database of Microbial Ecology Department and data for 2009-2011 from the present investigations. Mean values or single data points are shown with squares and standard deviations with thin lines. Lowess curves were fitted on the data.

The highest level of eutrophication took place in Lake Niegocin, which reached a persistently hypereutrophic status in the 1980s. Its surface waters were characterised by a mean TSI of 81 (SD 17) and TP of 435 µg L^{-1} (SD 153) during that time. A decrease in its trophic status began slowly from 1994 (figs 3 A and B). Mean TSI was 75 (SD 3), TP was 358 µg L^{-1} (SD 78), chl *a* was 16 µg L^{-1} (SD 28) and Secchi disk visibility was 1.0 m (SD 0.2) during 1990-1993. The mean values changed to 68 TSI (SD 8), 132 µg TP L^{-1} (SD 28), 16 µg chl *a* L^{-1} (SD 6) and 2.0 m Secchi disk visibility (SD 0.7) during 1994-2000. After 2000, the trophic state still continued to decrease and has remained stable at the border between mesotrophic and eutrophic classes. Mean TSI was 60 (SD 8), TP was 80 µg L^{-1} (SD 61), chl *a* was 21 µg L^{-1} (SD 9) and Secchi disk visibility was 1.9 m (SD 0.6) during 2001-2011.

In Lake Mikołajskie, oligotrophication was also apparent. The highest mean TSI and TP values recorded were 75 (SD 5) and 175 µg L^{-1} (SD 82), respectively, during 1980-1989. During the time period from 1991-2000, TSI decreased down to an average of 63 (SD 6) and TP down to 85 µg L^{-1} (SD 39, fig 3 C and D). The decrease continued after 2000. Mean TSI was 57 (SD 6) and TP was 43 µg L^{-1} (SD 23) during 2001-2011. However, chl *a* concentrations and Secchi disk visibility showed large variations without any observable trends. Mean chl *a* was 32 µg L^{-1} (SD 26) and Secchi disk visibility was 1.5 m (SD 0.4) during 1990-2011. Chl *a* concentration peaked at 137 µg L^{-1} in August 2010.

Lake Ryńskie followed a similar pattern of oligotrophication as lakes Niegocin and Mikołajskie. In Lake Ryńskie, mean TSI was 67 (SD 3) and TP was 79 µg L^{-1} (SD 13) during the 1980s.Trophic state was found to decrease during the 1990s: mean TSI was 65 (SD 6), TP was 100 µg L^{-1} (SD 27, fig 3 E and F), chl *a* was 37 µg L^{-1} (SD 13) and Secchi disk visibility was 1.2 m (SD 0.4). From 2000 to 2011, clear trends were not observed. Mean TSI was 59 (SD 5), TP was 44 µg L^{-1} (SD 19), chl *a* was 36 µg L^{-1} (SD 35) and Secchi disk visibility was 1.2 m

(SD 0.3). A considerable increase in chl *a* concentration was observed in 2010 (207 µg L^{-1} in August 2010).

Trends in changes of TP and chl *a* concentrations in the southern lakes during 1990-2011 were compared with a time series analysis. The concentrations were standardised [z = (x-mean)/SD] and smoothed (moving mean value over two points, fig 4).

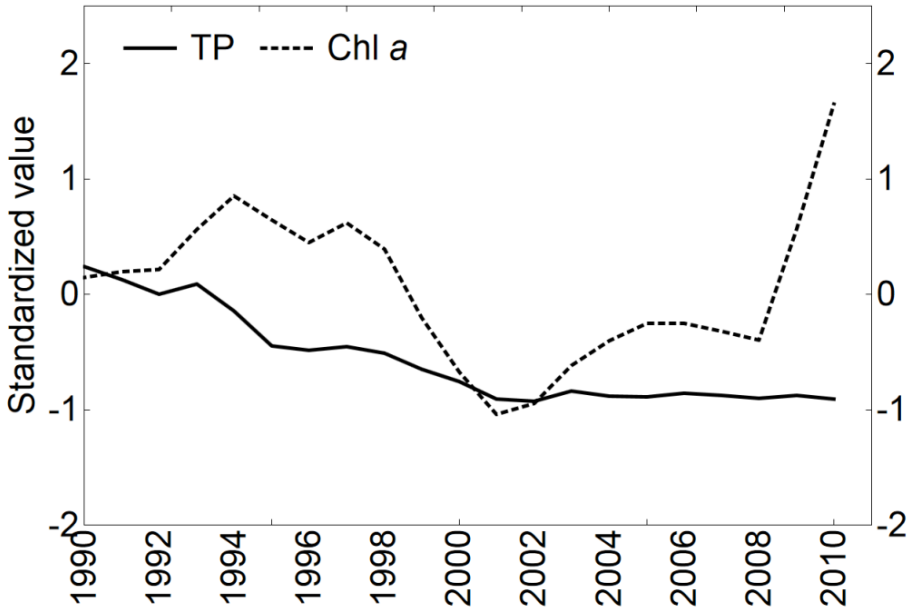

Fig 4. Time series of mean TP and chl *a* concentrations which were standardised [z = (x-mean)/SD] and smoothed (moving mean value over two points). Results shown from the southern lakes of the GML-system. Data were combined from the database of Microbial Ecology Department (1990-2008) and from results obtained during the present investigations (2009-2011).

TP and chl *a* concentrations in the southern lakes did not change at the same rate (fig 4). TP concentrations appear to have reacted faster to decreased external nutrient inputs than chl *a* concentrations. Also, TP concentrations may have already reached a new balanced state by 2000, while chl *a* was still demonstrating large variations.

Seasonal changes of TP concentrations in southern and northern lakes were analysed based on results obtained from the database of Microbial Ecology Department and the present investigations (fig 5). Only data from 2000-2011 were considered, because years before 2000

had much higher trophic states and their inclusion in the analysis increased the standard deviation considerably. Seasonal changes of TP concentrations in Lake Mikołajskie were analysed in more detail. Data were obtained throughout 2009 and 2010 (as part of non-routine investigations, fig 6).

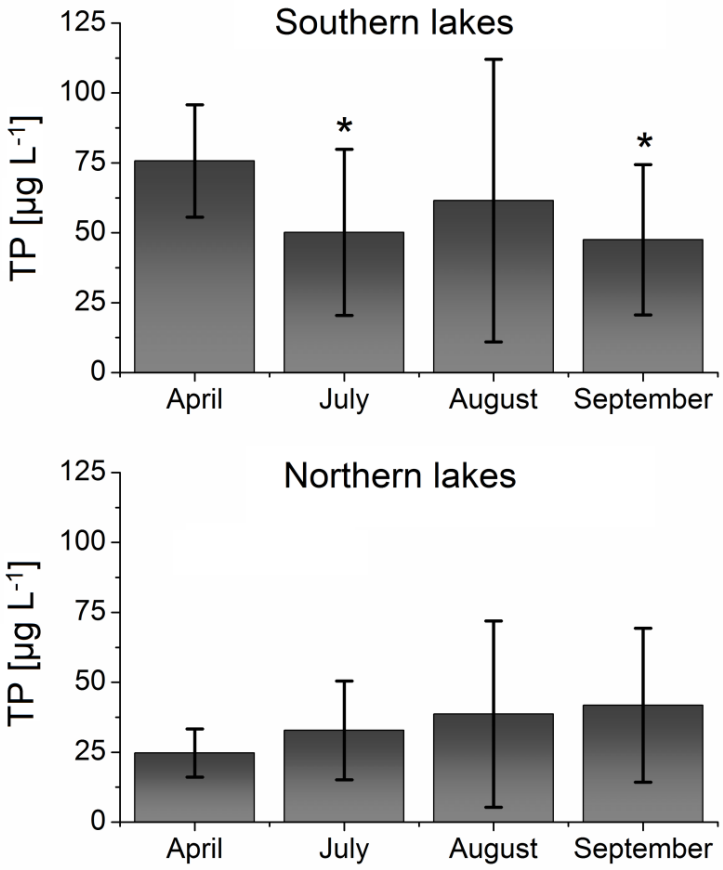

Fig 5. Seasonal means of limnetic TP concentrations during 2000-2011. Data for years 2000-2008 were obtained from database of Microbial Ecology Department, and data for 2009-2011 from the present investigations. Column height indicates mean values and thin lines show standard deviation. Stars show statistically significant changes in comparison to April values (Welch's t-test).

Significant changes in mean limnetic TP concentrations occurred in the southern lakes between spring and summer seasons of 2000-2011 (fig 5). The means were highest in April and generally decreased during summer months. An exception was August, when high variability

was observed. In the northern lakes, means of limnetic TP concentrations remained at similar levels throughout spring and summer seasons.

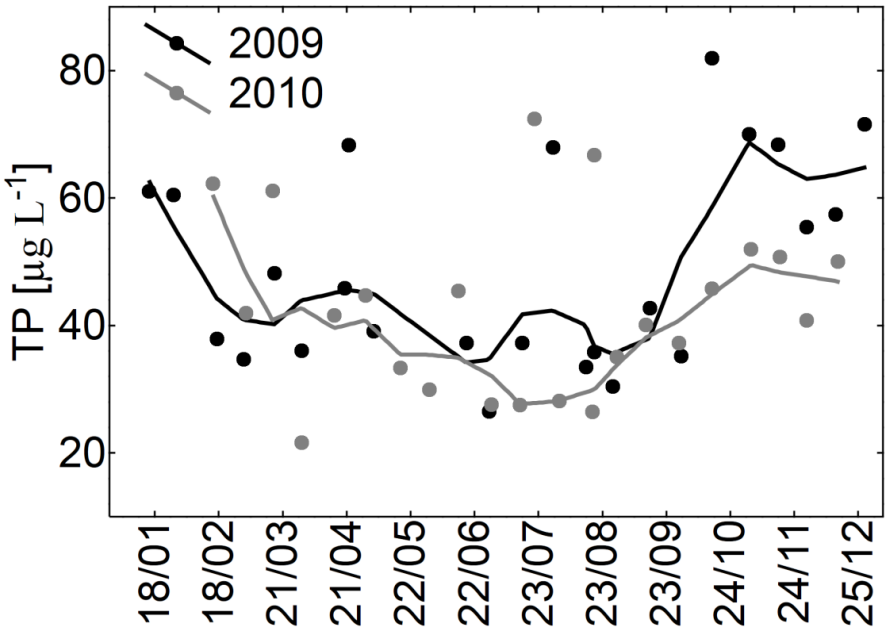

Fig 6. Total phosphorus (TP) concentrations in limnetic zone of Lake Mikołajskie during 2009 and 2010. Lowess curves were fitted on the data.

In Lake Mikołajskie, the highest TP concentrations in the limnetic zone occurred generally during winter, spring and autumn seasons and the lowest concentrations were observed during summer months (fig 6).

Present state of the GML-system

In all of the following paragraphs, only data obtained from present investigations carried out during summer seasons of 2009-2011 were included. Quite different ecological conditions prevail during the spring, and overturn in comparison to the stratified stagnation period of the summer, which makes the seasons not directly comparable to each other.

Means and standard deviations of TSI (fig 7), ratio of thickness of hypoxic column depth to maximum depth of a lake at sampling location (fig 8) and profundal TP concentrations (fig 9) in each lake of the GML-system during 2009-2011 were calculated.

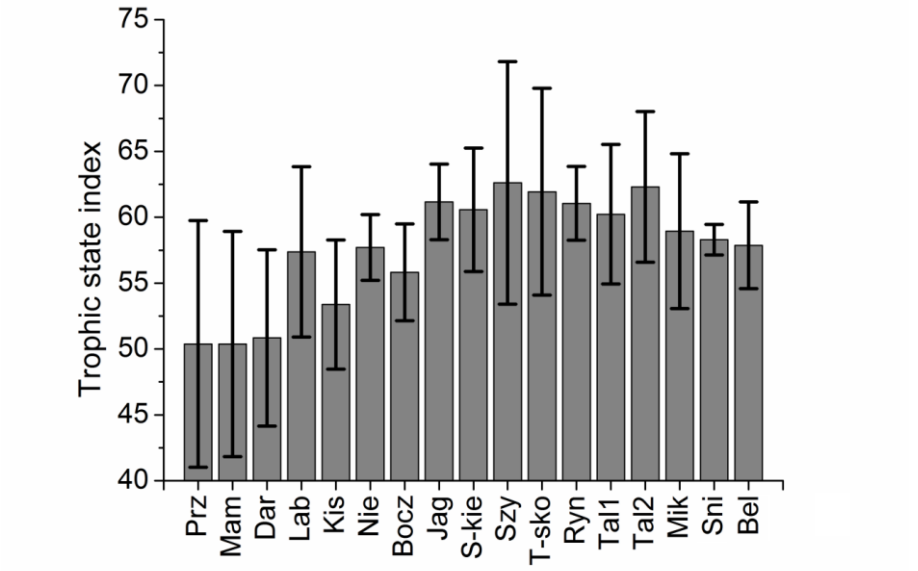

Fig 7. Mean trophic state index of lakes of the GML-system during 2009-2011. Standard deviation is shown by thin lines. Lakes have been organised according to their geographical order—from north to south.

The highest mean TSIs (range between 60 and 63) were in the southern lakes Jagodne, Szymoneckie, Szymon, Tałtowisko, Ryńskie and Tałty based on data from 2009-2011 (fig 7). In the rest of the lakes, mean TSIs were between 50 and 60. Thus, based on the mean TSI values from 2009-2011, all of the investigated lakes could be classified as eutrophic.

High increases in TSI and other associated parameters occurred in 2010. In all of the studied lakes, TSIs ranged from 54 to 70 in July 2010 and from 60 to 74 in August 2010. This caused large standard deviation between years, which was generally higher in the northern than southern lakes. Otherwise, TSIs ranged from 42 to 61 in 2009 and from 44 to 60 in 2010, which are within the range from mesotrophy (lakes Przystań, Mamry and Dargin) to eutrophy (the remaining lakes).

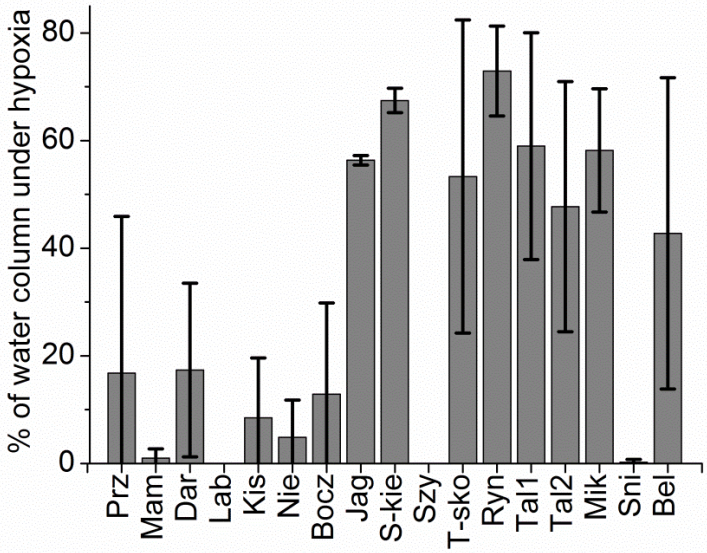

Fig 8. Proportion of thickness of hypoxic water column to maximum depth of each lake at sampling sites during 2009-2011. Column heights indicate mean values, thin lines denote standard deviation. No signs of hypoxia were observable in lakes Łabap and Szymon.

Hypoxia developed persistently in the majority of the deep southern lakes of the GML-system during the summer months of 2009-2011 (lakes Jagodne, Szymoneckie, Tałtowisko, Ryńskie, Tałty sites 1 and 2, Mikołajskie and Bełdany; fig 8). In the northern lakes and polymictic southern lakes (Niegocin, Boczne and Śniardwy), hypoxia did not generally develop. A notable exception was August 2010, when all lakes except Przystań, Łabap and Szymon developed hypoxia. Only lakes Łabap and Szymon did not show signs of hypoxia.

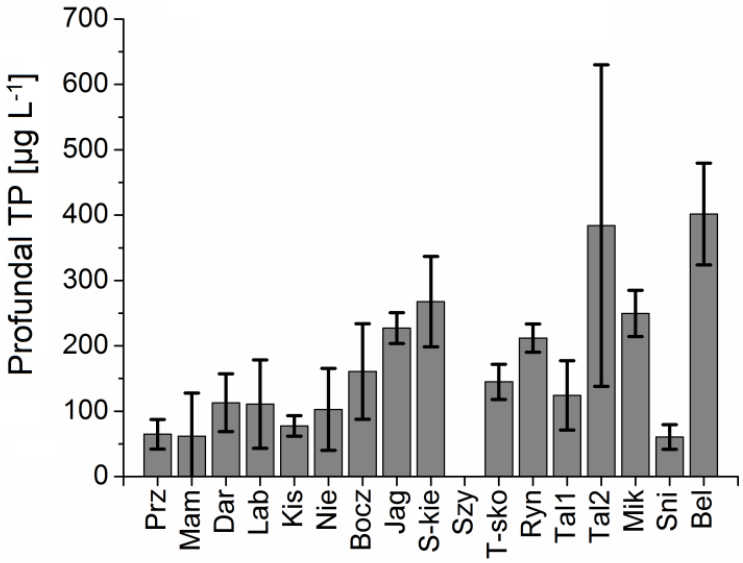

Fig 9. Profundal concentrations of total phosphorus (TP, mean values indicated by column height) during the summers of 2009 and 2010 had high standard deviation (thin bars). For comparison, mean TP of the limnetic zone in whole GML-system was 50 µg L^{-1}.

Mean profundal TP concentrations were higher than 200 µg L^{-1} in lakes Jagodne, Szymoneckie, Ryńskie, Tałty site 2, Mikołajskie and Bełdany during the summers of 2009 and 2010 (fig 9). Values higher than 300 µg L^{-1} were observed in lakes Szymoneckie, Tałty 2 and Bełdany, but not repetitively.

Biomass of algae and cyanobacteria and number of heterocysts were analysed in 2010 and 2011 (figs 10 and 11).

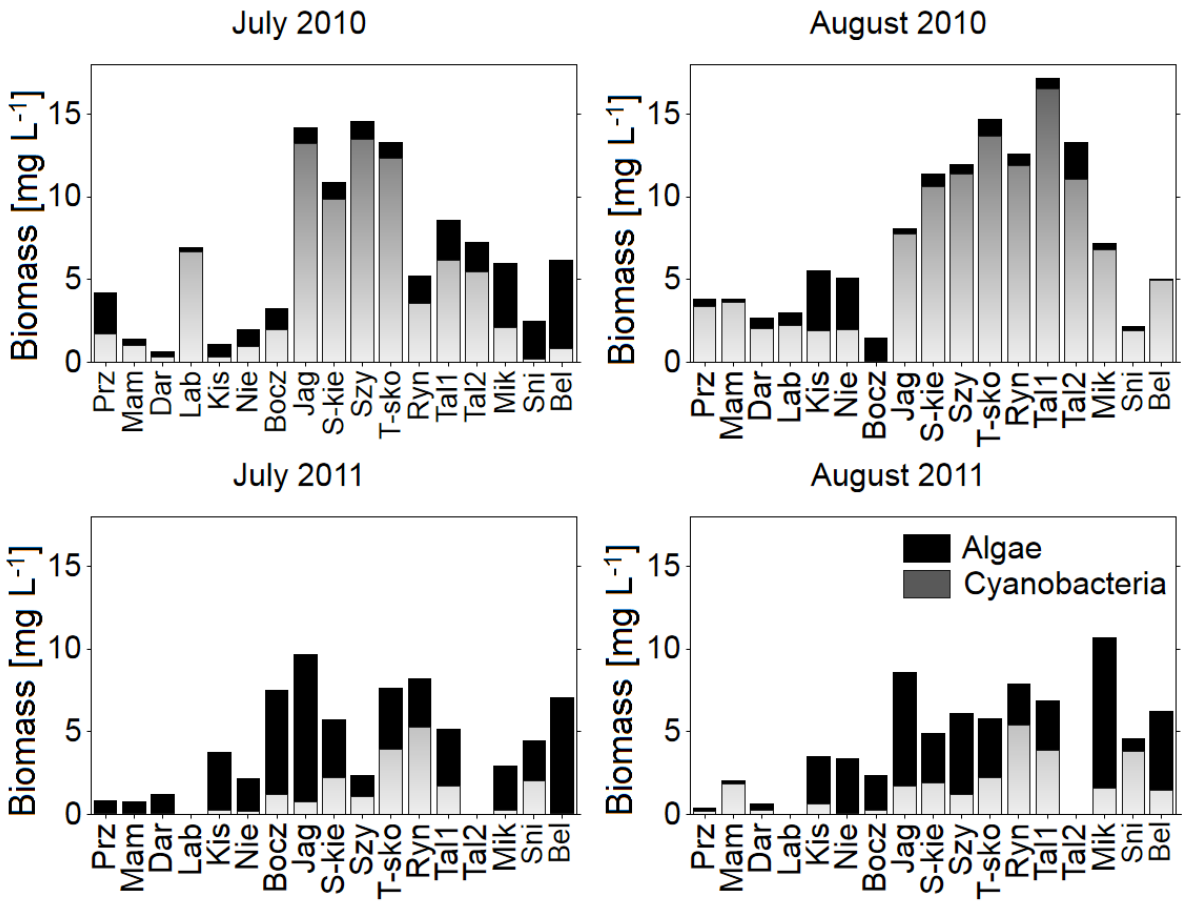

Fig 10. Biomasses of algae and cyanobacteria in 2010 and 2011.

Phytoplankton community was heavily dominated by cyanobacteria in 2010 (mean percentage of cyanobacteria from whole phytoplankton biomass was 70% in all lakes, SD 27; fig 10). In 2011, the percentage of cyanobacteria decreased (mean 31%, SD 25) and also total phytoplankton biomasses were lower. Generally, phytoplankton biomass was higher in the southern dimictic lakes and Szymon than in the northern lakes and in the rest of the southern lakes (Niegocin, Boczne, Śniardwy).

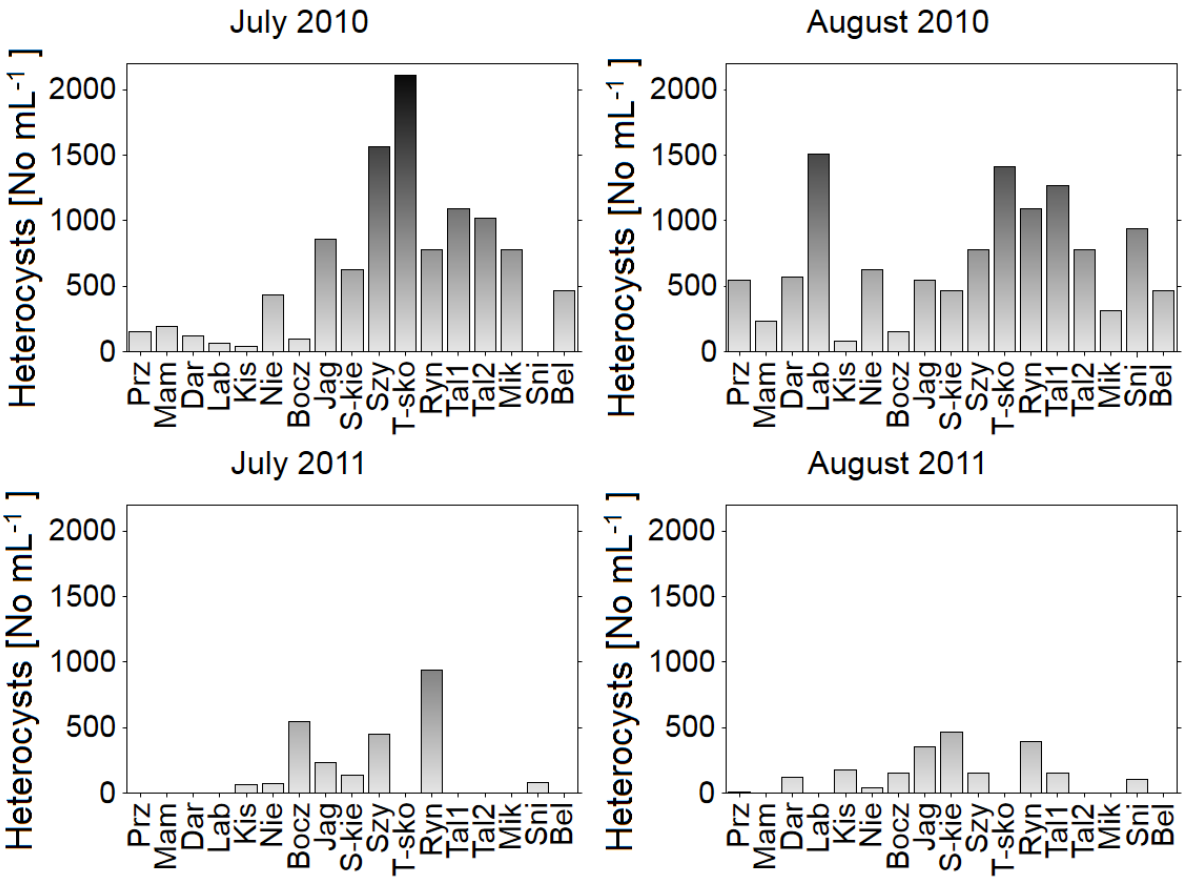

Fig 11. Number of heterocysts in 2010 and 2011.

The number of heterocysts was significantly higher in 2010 (mean 654 mL^{-1}, SD 511) than in 2011 (mean 160 mL^{-1}, SD 220, fig 11), but also highly variable between lakes (fig 11). The number of heterocysts had a strong positive correlation with the biomass of cyanobacteria (R^2 0.51).

External nutrient inputs

Data about inorganic fertiliser usage were collected from external databases (SOS 1992-1996, BDL 2012). Data was available at region (voivodeship) level. The division of Poland into regions changed in 1999: earlier, the GML-system was part of the Suvalskie province but

afterwards it belonged to Warmia-Masuria. The geographical borders of these areas are different, thus the fertiliser data are not directly comparable between years.

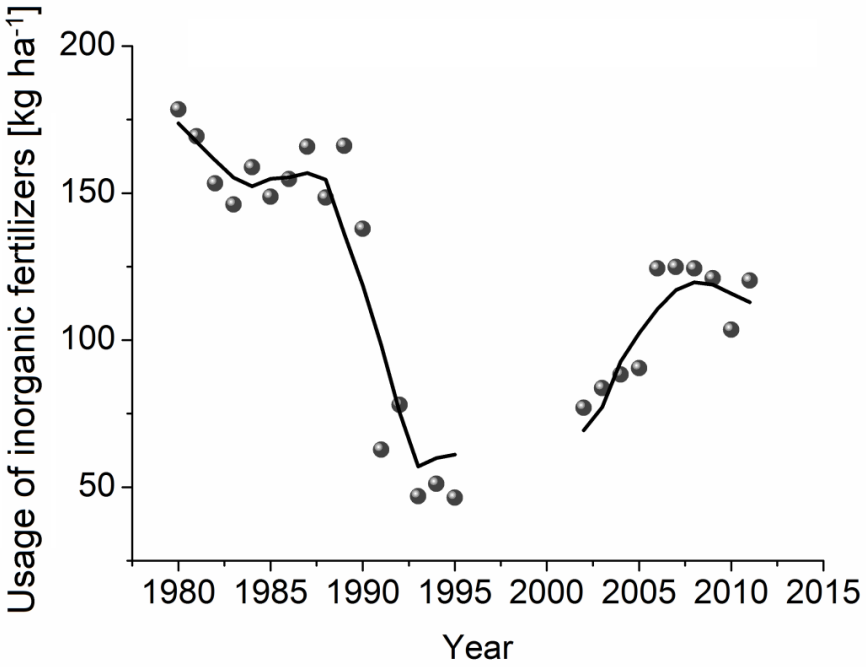

Fig 12. Usage of inorganic fertilisers in agriculture in the region of the GML-system. Data from years 1980-1995 were from Suvalskie province (SOS 1992-1996) and data from years 2002-2011 from Warmia-Masuria province (BDL 2012). Lowess-smoothed trend line was fitted to data.

The consumption of inorganic fertilisers decreased rapidly in 1990-1991 (fig 12). After the drop, the amount of applied inorganic fertilisers per hectare was about one third of previous values. Fertiliser usage since 2002 has almost doubled.

The agricultural area in Warmia-Masuria province decreased slightly between 2004 and 2009, when 44.6% and 42.9% of all land was in agricultural use, respectively (BDL 2012).

Nutrient loads (kg m^{-2} $year^{-1}$) of lake area from wastewater treatment plants to lakes Niegocin, Mikołajskie and Ryńskie were calculated based on total nutrient load data (kg $year^{-1}$) obtained from BDL (2012) and lake area data from Waluga and Chmielewski (1998, 1999), and Jańczak (1999).

Table 3. Means values of total nutrient load from wastewater treatment plants in Giżycko, Ryń and Mikołajki. Yearly data available from BDL (2012).

Mean from years	Town	Phosphorus load [kg year^{-1}]	SD	Nitrogen load [kg year^{-1}]	SD
1998-2011	Giżycko	1337	514	21670	6456
	Ryn	911	483	5491	3778
	Mikołajki	288	117	5794	1439
2009-2011	Giżycko	958	39	20048	3361
	Ryn	805	660	2453	801
	Mikołajki	176	30	5455	974

Table 4. Mean values of nutrient load per square metre of lake area of the receiving lakes Niegocin, Mikołajskie and Ryńskie. Results calculated based on nutrient load values provided by BDL (2012) and lake area obtained from Waluga and Chmielewski (1998, 1999), and Jańczak (1999).

Mean from years	Lake	Phosphorus load [kg m^{-2} year^{-1}]	SD	Nitrogen load [kg m^{-2} year^{-1}]	SD
1998-2011	Niegocin	51	20	833	248
	Ryńskie	135	71	5491	3778
	Mikołajskie	58	24	1164	289
2009-2011	Niegocin	37	2	771	129
	Ryńskie	119	98	2453	801
	Mikołajskie	35	6	1095	196

According to data available from BDL (2012), the total nutrient load from wastewater treatment plant in Mikołajskie decreased after 2006 (when the plant was partially modernised). Mean nutrient loads were 327 kg-P year^{-1} (SD 101) and 6202 kg-N year^{-1} (SD 1530) from 1998-2006. The mean load was lower if a longer time period was taken into account. Average loads from 2007-2011 were 219 kg-P year^{-1} (SD 33) and 5039 kg-N year^{-1} (SD 1152). The wastewater treatment plant Giżycko went through some changes in 2003, which caused a decrease in nitrogen loading. Mean nutrient loads were 1205 kg-P year^{-1} (SD 638) and 26350 kg-N year^{-1} (SD 8556) from 1998-2002. The mean loads changed to 1410 kg-P year^{-1} (SD 457) and 19070 kg-N year^{-1} (SD 3138) during 2003-2011 (BDL 2012).

Generally, means of total nitrogen and phosphorus loads (kg-N year^{-1} and kg-P year^{-1}) from the treatment plant in Giżycko based on data from 1998-2011 were four times higher than

the load from the wastewater treatment plant in Mikołajki (table 3, BDL 2012). The phosphorus load originating from Ryn's plant was almost equal to the load from the plant in Giżycko. When the total load was calculated as a mean load over the whole receiving lake area (kg-P m^{-2} $year^{-1}$), it was observed that the phosphorus load Lake Ryńskie received was much higher than the loads to lakes Niegocin or Mikołajskie (table 4). The means of phosphorus loads were lower in Niegocin and Mikołajskie during 2009-2011 than during 1998-2011, indicating a decreased load from wastewater treatment plants. No similar changes occurred in phosphorus load to Lake Ryńskie.

Mean TN:TP ratio of the treated sewage was highest in the treatment plants in Mikołajki and Giżycko, which have tertiary treatment. They had a mean TN:TP ratio of 23:1 (SD 8:1) and 18:1 (SD 8), respectively, during years 1998-2011 (calculated based on data from BDL 2012). The plant in Ryn only has biological treatment and had a mean TN:TP ratio of 8:1 (SD 6).

Chloride and sulphate concentrations were analysed during the summers of 2009 and 2010 as a part of present non-routine investigations. The arithmetic mean of the two concentrations was calculated for each lake and used to evaluate anthropogenic pressure in the GML-system.

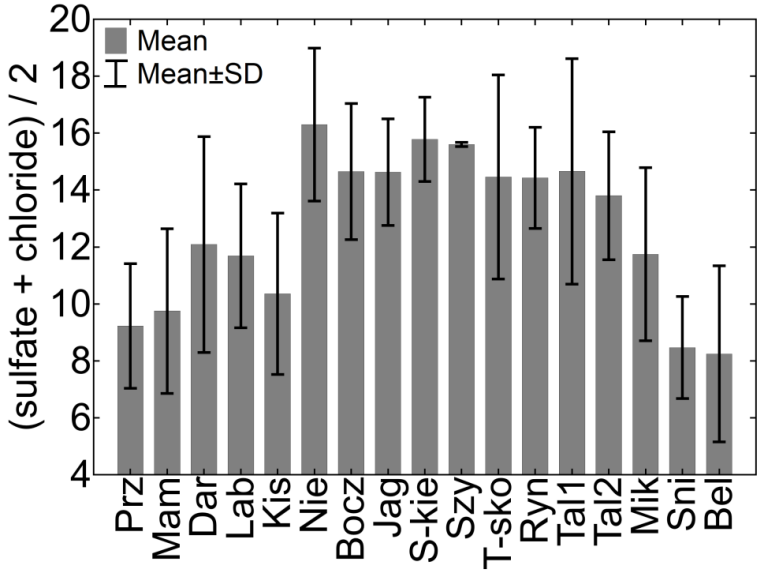

Fig 13. Anthropogenic pressure estimated as a mean of sulphate and chloride concentrations in the limnetic zone of lakes of the GML-system during 2009 and 2010.

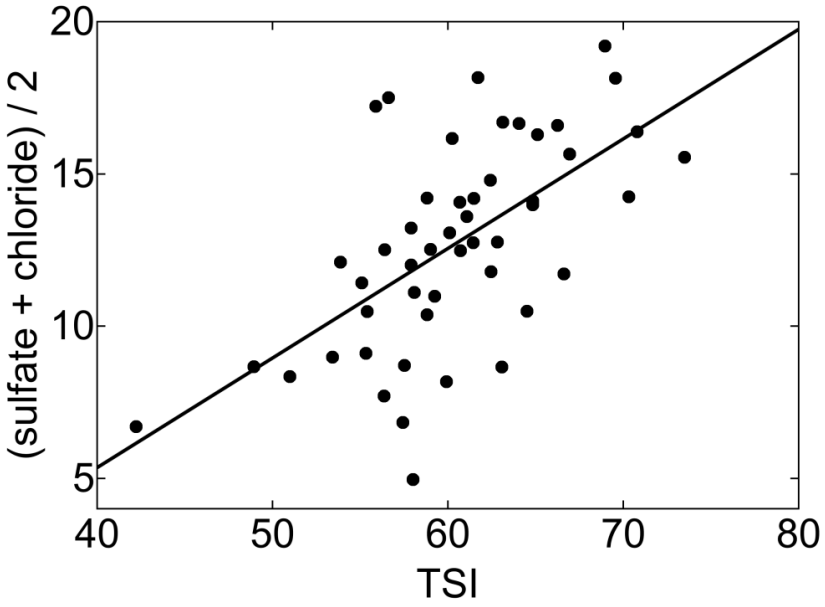

Fig 14. Relationship between anthropogenic pressure estimated as a mean of chloride and sulphate concentrations and trophic state index (R^2 0.26).

The arithmetic mean of chloride and sulphate from 2009 and 2010 were highest in lakes Niegocin, Boczne and Jagodne (fig 13) and decreased towards the other southern lakes. The mean value had a strong positive correlation with TSI (R^2 0.26, fig 14).

Internal nutrient cycles and microbial activity

Rates of primary production and respiration were calculated for a 24 hour period, assuming a 12/12 hour light/dark cycle. The ratio of gross primary production (GPPr) to respiration rate (Rr) was counted. Results of GPPr and Rr in fig 15 were obtained from analyses of oxygen concentration changes measured with light-dark bottle methods (Carpenter 1965, Howarth and Michaels 2000, Pace and Prairie 2005). The analyses were carried out during 2009 and 2010. In fig 16, measurements of Rr were obtained from analyses using the modified method (Kauppinen et al. 2013) and GPPr using the C^{14}-method (Chróst and Siuda 2006). The analysis of Rr with the modified method was carried out during 2010 and 2011.

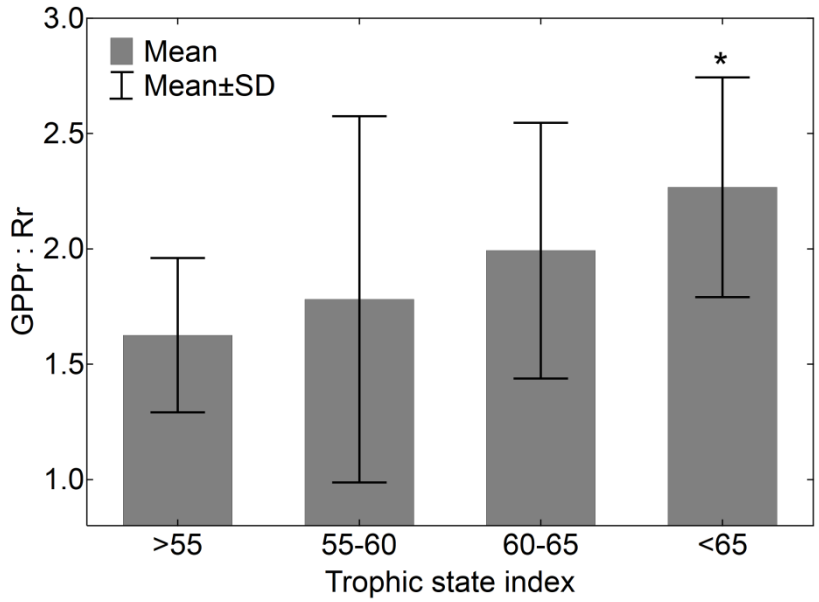

Fig 15. Change in ratio of gross primary production rate (GPPr) to respiration rate (Rr) with increasing trophic state. Results were calculated assuming a 12/12 hours dark/light cycle and determined using a classical light-dark bottle method (Howarth and Michaels 2000, Pace and Prairie 2005). A star above a column indicates a statistically significant difference in comparison to category TSI>55.

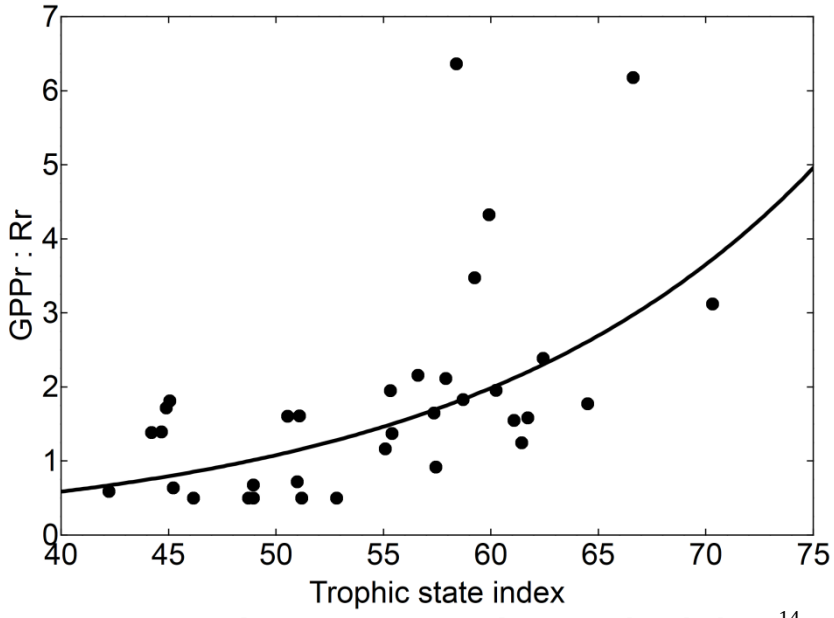

Fig 16. Ratio of gross primary production rate (GPPr, determined with the C^{14} method by Chróst and Siuda 2006) to respiration rate (Rr, determined with the modified method by Kauppinen et al. 2013) in comparison to trophic state index ($R^2 = 0.37$).

The ratio of GPPr to Rr increased with TSI in the GML-system, in a manner that was not dependent on the methodology used (figs 15 and 16). Both methods gave results in the same range of values, although they cannot be directly compared due to differences in the timing of when the results were obtained. Based on light-dark bottle methods, the GPPr:Rr ratio had a mean value of 1.6 when TSI was below 55 and increased to 2.3 when TSI was above 65 (fig 15). Based on the modified dark bottle method and the C^{14}-method, GPPr:Rr had an exponential relation with TSI ($R^2 = 0.37$), and the ratio ranged from an average of 1 when TSI was below 50 up to 2.5 when TSI was above 60 (fig 16).

Differences in Rr (measured with classical dark bottle method), enzymatic activity (AMP and APA), net primary production (NPPr, measured with C^{14} method) and percentage of extracellular release (PER) between mesotrophic and eutrophic lakes of the GML-system were compared and tested with Welch's t-test. All results were obtained as part of the present routine investigations during 2009-2011.

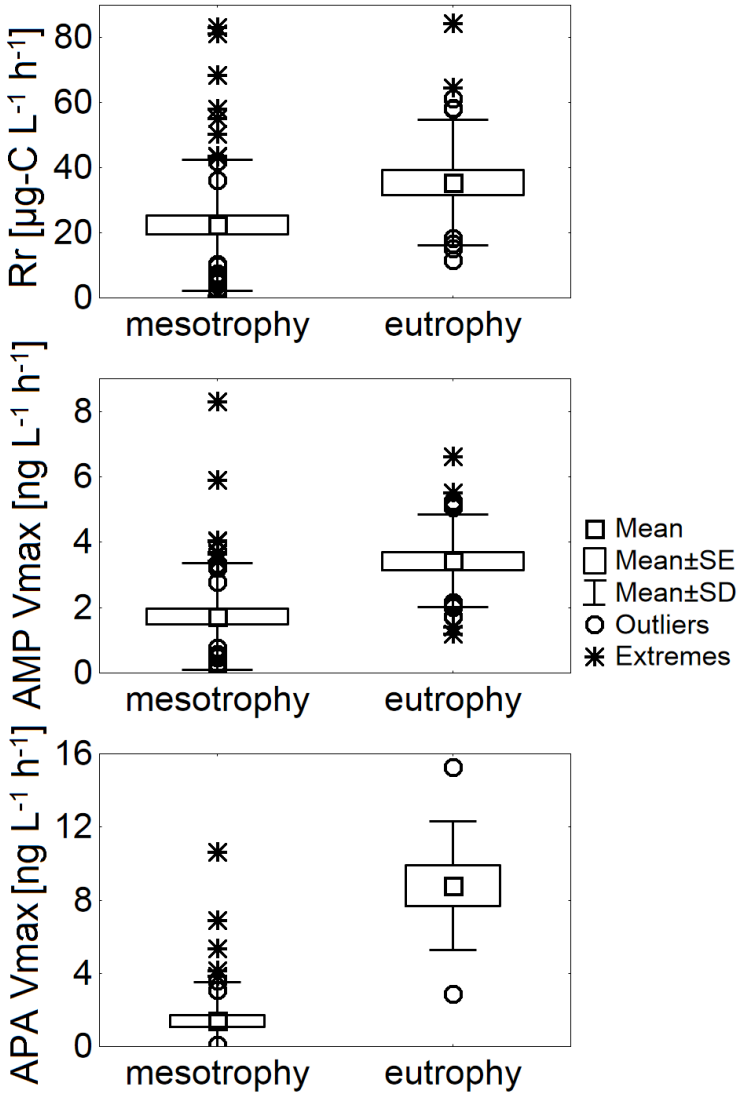

Fig 17. Respiration rate (Rr), L-leucine aminopeptidase (AMP Vmax) and alkaline phosphatase (APA Vmax) activities at different trophic states.

Rr, AMP and APA activities showed statistically significant increases in eutrophic environments in comparison to mesotrophic environments (fig 14). No significant differences in NPPr and PER were found between mesotrophic and eutrophic environments (results not shown).

Mean values and standard deviations of Rr, enzymatic activity (AMP and APA), NPPr and PER were calculated for each studied lake of the GML-system (figs 18 and 19).

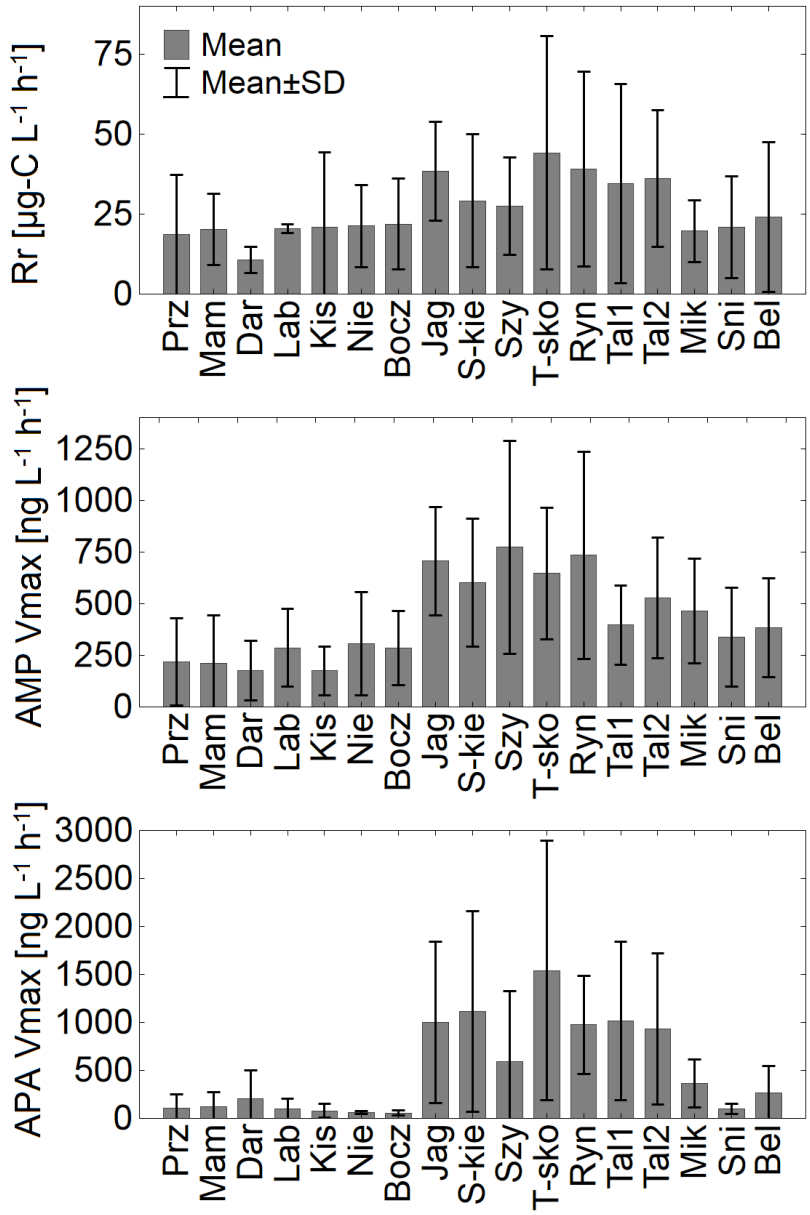

Fig 18. Mean respiration rate (Rr, measured with classical dark bottle method), L-leucine aminopeptidase (AMP Vmax) and alkaline phosphatase (APA Vmax) activities in the GML-system during the summer months of 2009-2011.

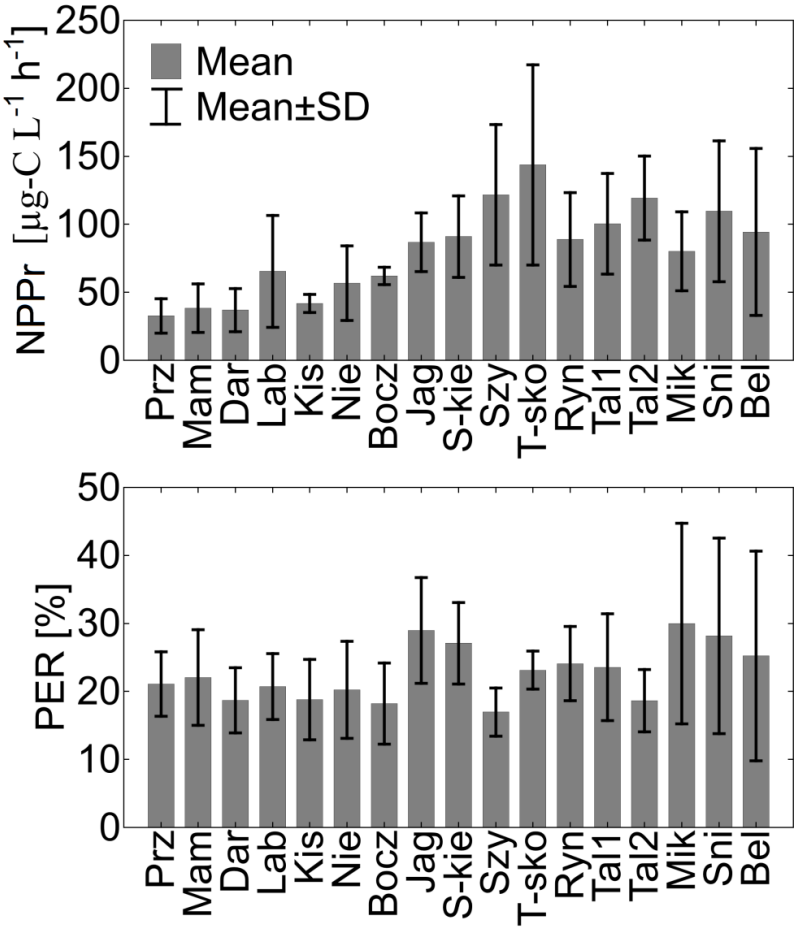

Fig 19. Means of the rate of net primary production (NPPr) and percentage of extracellular release (PER, measured with the ^{14}C-method) in the GLM-system during the summer months of 2009-2011.

AMP and APA activities had the highest differences between the lakes of the GML-system (fig 18). The AMP and APA lowest activities occurred in the northern drainage area and in lakes Niegocin, Boczne, Mikołajskie, Śniardwy and Bełdany. A similar pattern was observed in Rr, but the differences between lakes were smaller. NPPr and PER had high standard deviation between years and PER in particular did not show any identifiable trend between the lakes (fig 19).

Nutrient limitation

Information about changes in TN:TP ratio with time were gathered from the database of Microbial Ecology Department (2004 and 2005), results obtained during the monitoring (2009-2011) and a publication by Kufel (1984-1996, Kufel 2001). The amount of obtainable data was limited due to low number of simultaneous TN and TP measurements in the GML-system.

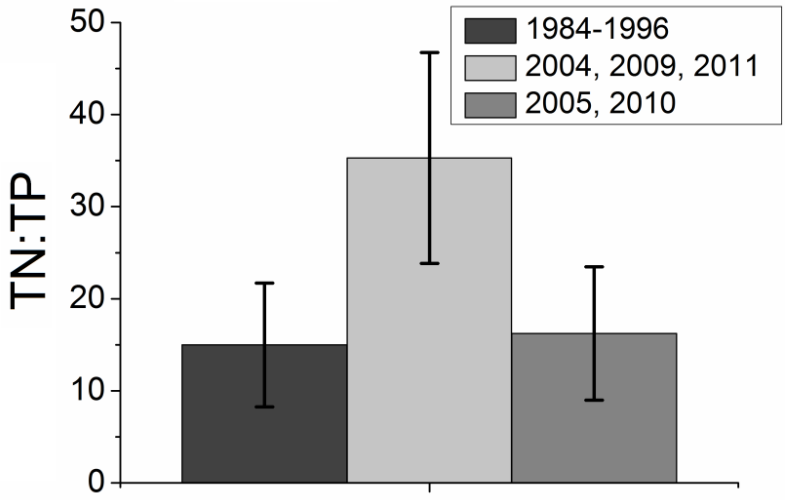

Fig 20. Mean ratio between total nitrogen and total phosphorus (TN:TP) during different time periods, with standard deviation. Results for 1984-1996 were from Kufel (2001), 2004-2008 from the database of the Microbial Ecology Department and 2009-2011 from the present investigations. Standard deviations are shown by thin lines.

TN:TP ratios calculated from TN and TP concentrations published by Kufel (2001) showed that the ratios were low (mean 15:1) during 1984-1996 (fig 20). Since then, TN:TP ratios appear to have increased: the mean ratio was about 35:1 in 2004, 2009 and 2011. However, in 2005 and 2010, the TN:TP ratio was low, with an average of 16:1.

Changes in nutrient limitation during 2009-2011 were investigated in more detail. TN, TP and chl *a* concentrations were $\log_{10}$-transformed and correlations were analysed for each year separately (fig 21); the transformed values were compared to Dillon and Rigler's ratio (1974; fig

22). TN:TP ratio was plotted against TSI and a linear regression analysis was carried out (fig 23).

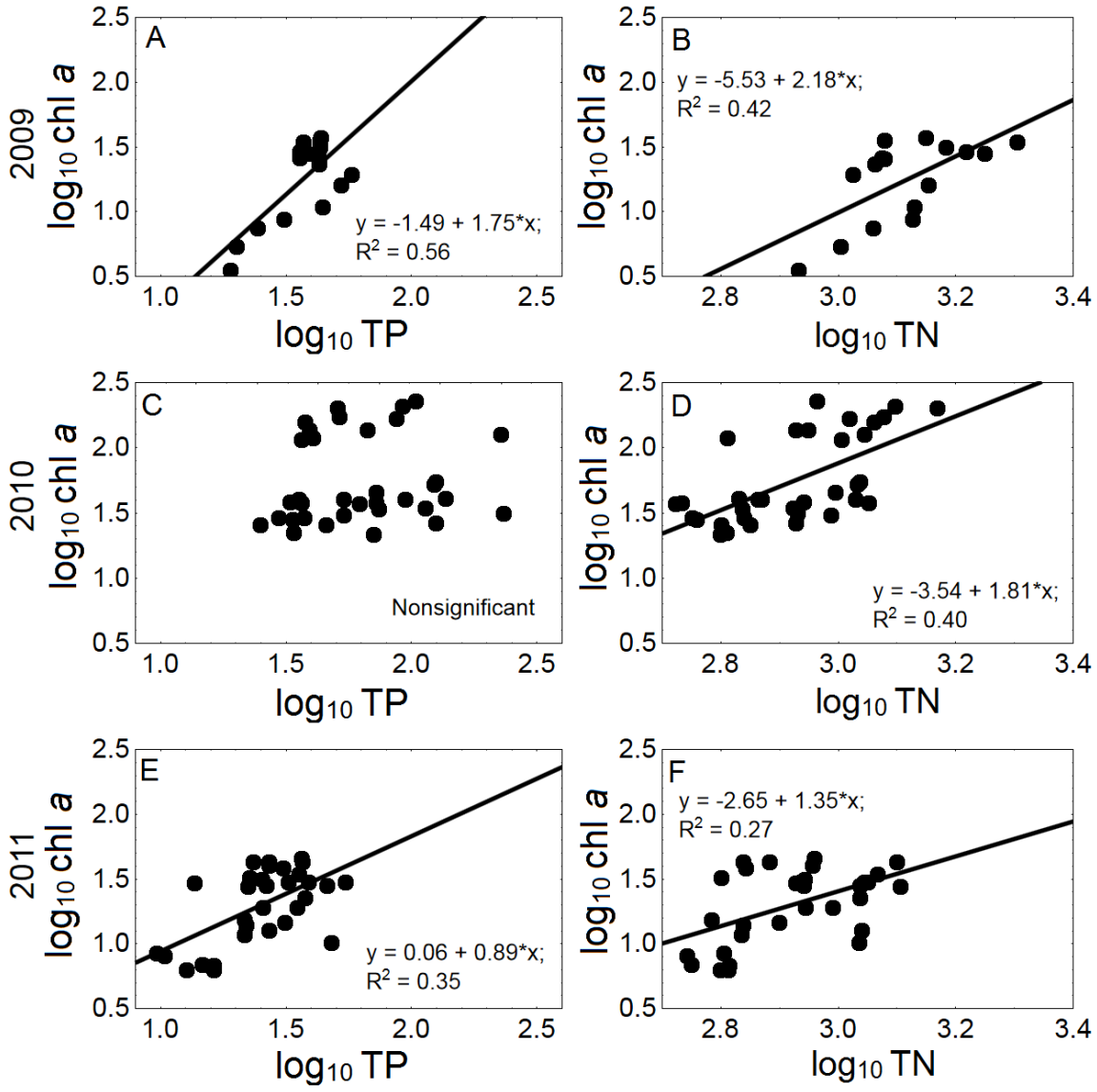

Fig 21. Correlations between $\log_{10}$ -transformed nutrient (total phosphorus - TP and total nitrogen - TN) and chlorophyll *a* (chl *a*) concentrations in the GML-system during 2009 (A, B), 2010 (C, D) and 2011 (E, F). All data were collected during present investigations.

Comparison of $\log_{10}$-transformed nutrient and chl *a* data during each separate year when monitoring was carried out, showed that chl *a* had a strong or moderate positive correlation with TN and TP in both 2009 and 2011 (fig 21). However, in 2010, chl *a* was only correlated with TN.

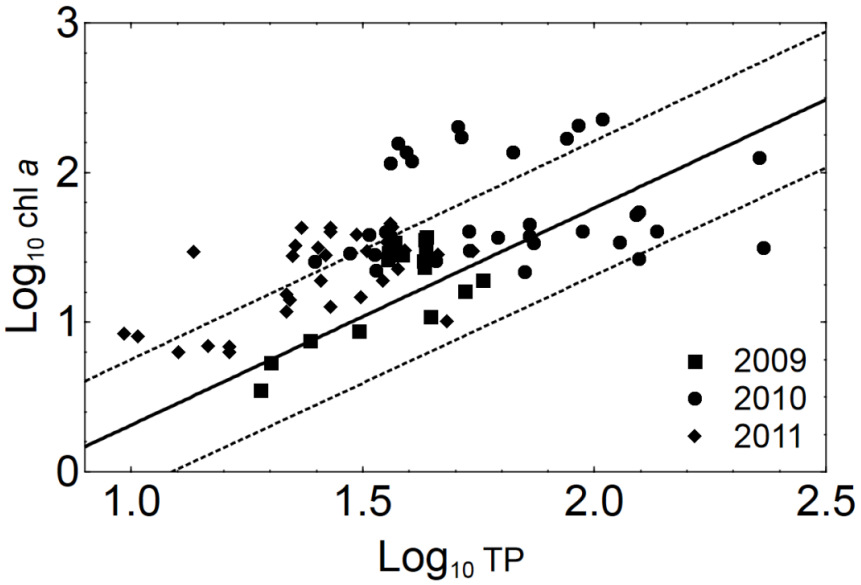

Fig 22. Plotted log_{10} transformed total phosphorus (TP) and chlorophyll *a* (chl *a*) concentrations against Dillon and Rigler's ratio (solid line, dashed lines show 0.95 confidence intervals for the ratio; Dillon and Rigler 1974).

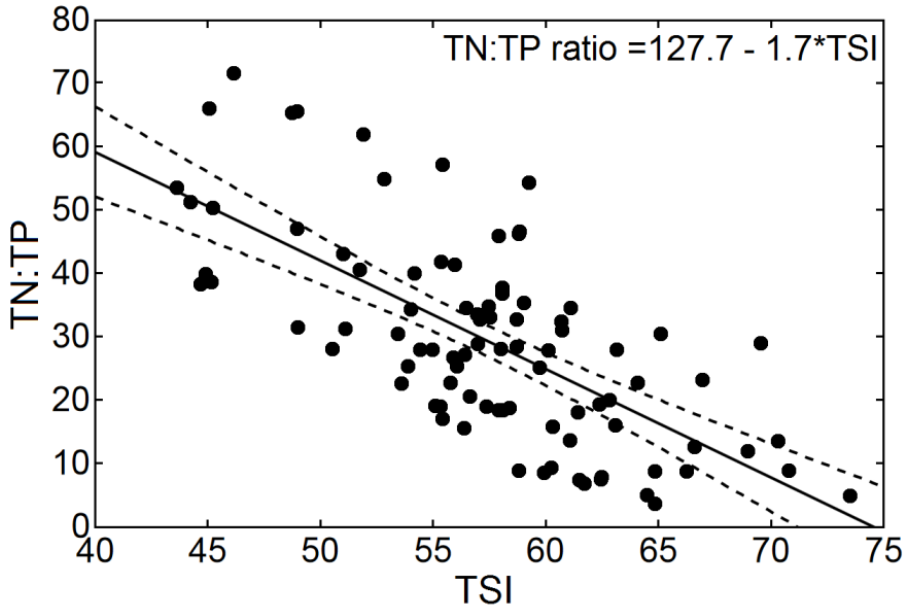

Fig 23. Linear regression of the relationship between the ratio of total nitrogen to total phosphorus (TN:TP) and trophic state index (TSI) in the GML-system based on results obtained during the present investigations during 2009-2011 (0.95 confidence intervals - dashed lines, R^2 0.44, standard error 10.8, residuals follow normal distribution).

Comparison of log_{10}-transformed chl *a* and TP results to Dillon and Rigler's ratio (1974), showed that plotted results were generally above the ratio line and, in many cases, even outside the 0.95 confidence limits (fig 22).

A linear regression model (TN:TP ratio = 127.7 - 1.7*TSI) was found to describe the strong negative relationship between TN:TP ratio and TSI (R^2 0.44, fig 23). Residuals from the model followed normal distribution and the standard error of predicted results was 10.8.

In an attempt to describe how the biomass of cyanobacteria is regulated in the GML-system, a model to describe the relationship between this and environmental variables was constructed. A forward stepwise multiple regression analysis was performed on available appropriate data, with cyanobacterial biomass as a dependent variable. Chl *a* concentration was excluded from the analysis because it was strongly correlated with cyanobacterial biomass. The test entered the variables TSI, TN:TP ratio and algal biomass into the model. All entered predictors showed a significant contribution to the model. The rationale behind how the entered predictors explained the variation in biomass of cyanobacteria was visually verified with principal components analysis (fig 24). The analysis included the variables cyanobacterial biomass and TSI to factor 1, and algal biomass and TN:TP ratio to factor 2, which showed that majority of variability within biomass of cyanobacteria should be taken into account by the model variables.

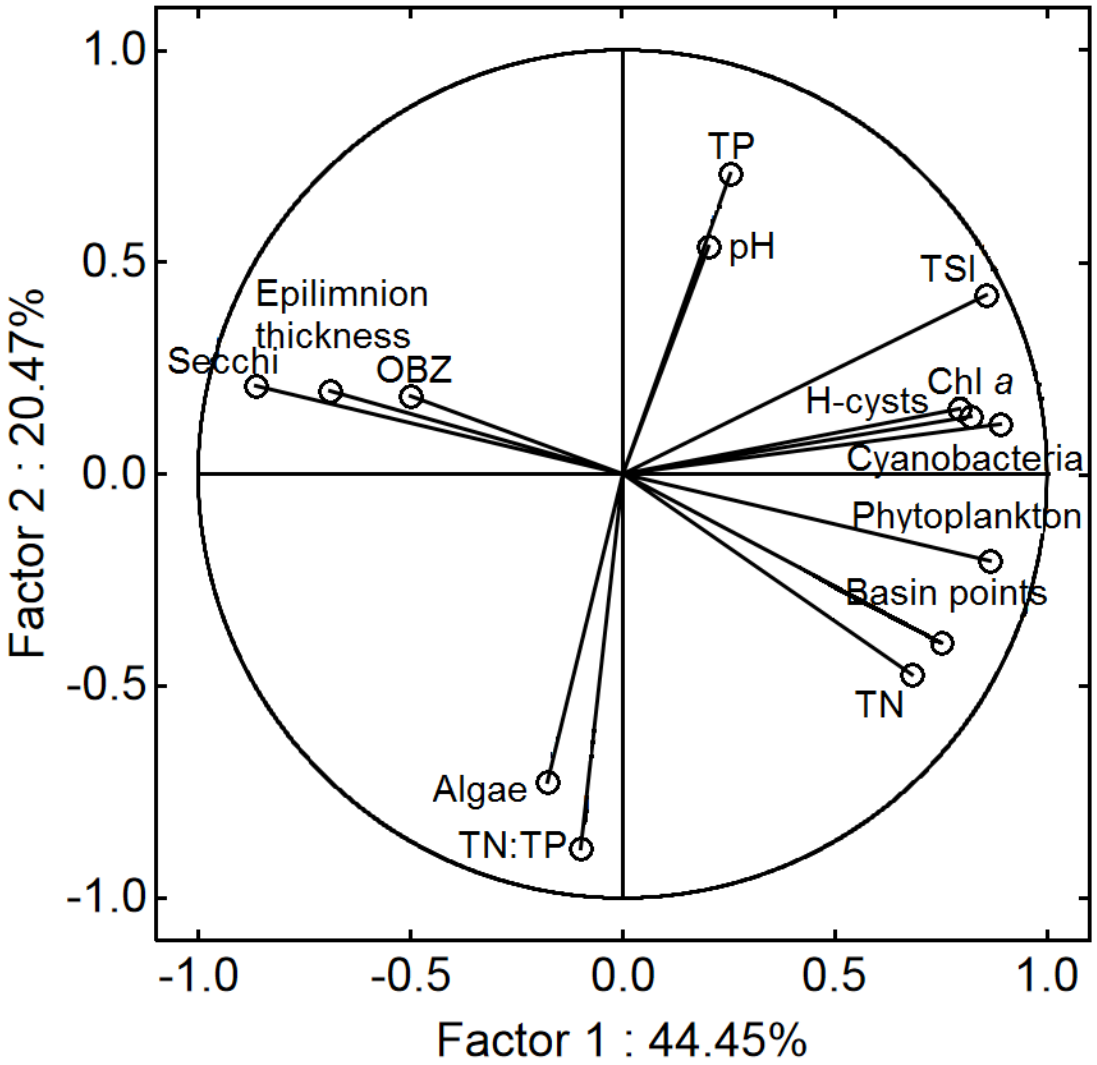

Fig 24. Principal components analysis showing relative importance of variables in explaining variance in factor components. A variable's distance from the centre of the circle is equal to its correlation with factor components (i.e. distance varies between -1 and 1). The analysis provides a visual scale of how variables are grouped by the factor components. Factor 1 explained approximately 43% of total variance and factor 2 about 19%. Explanations for abbreviations: TP - total phosphorus, TN - total nitrogen, TN:TP - ratio of TN to TP, Secchi - Secchi disk visibility, Chl *a* - chlorophyll *a*, TSI - trophic state index, H-cysts - number of heterocysts, OBZ - thickness of oxygenated buffer zone. Cyanobacteria, phytoplankton and algae refer to their respective biomasses.

Cyanobacterial biomass was identified to follow log-normal distribution; thus, a new multiple regression analysis was performed on these variables after log-transformation using all effects. Based on these results, the log-transformed prediction of cyanobacterial biomass can be calculated from the equation:

$$Log_{10}(Biomass\ of\ cyanobacteria) =$$

$$-24.54 + 13.26*\log_{10}(TSI) + 1.09*\log_{10}(algal\ biomass) - 0.39*\log_{10}(TN{:}TP\ ratio)$$

In which, *algal biomass* stands for eukaryotic phytoplankton biomass (mg L^{-1}), *TSI* for mean trophic state index (mean of trophic state indexes based on TP, chl *a* and Secchi disk visibility, following Carlson 1977) and *TN:TP ratio* for the ratio between total nitrogen and total phosphorus concentrations (in weight). Prediction of cyanobacterial biomass [mg L^{-1}] can be calculated through the inverse of $\log_{10}$ (namely 10^x). The coefficient of multiple regression (R^2) was 0.64, standard error of final predictions was 2.5 (or of log-transformed prediction 0.4, table 5) and degrees of freedom were 36.

Table 5. Multiple regression results for log-transformed cyanobacterial biomass. Coefficients b* (standardised coefficients, for comparison of contribution of each predictor to the final prediction) and b (used for predicting values) and their standard errors (SE). R^2 of the multiple determination was 0.64, F(3,60)=36.0, standard error of estimate 0.4 and N=64.

	b*	**SE of b***	**b**	**SE of b**
Intercept			-24.54	2.67
Log (TSI)	1.03	0.11	13.26	1.38
Log (Algal biomass)	0.47	0.11	1.09	0.26
Log (TN:TP ratio)	-0.27	0.08	-0.39	0.12

The model verification was done by analysis of residuals (i.e. the difference between predicted and observed results). The residuals showed normal distribution (fig 25). The predicted biomass of cyanobacteria had a moderately good fit with the observed biomass values, but the fit decreased with higher values (slope = 0.84, fig 26). A low amount of observed results from high cyanobacterial biomass values decreased the predictive power of the model.

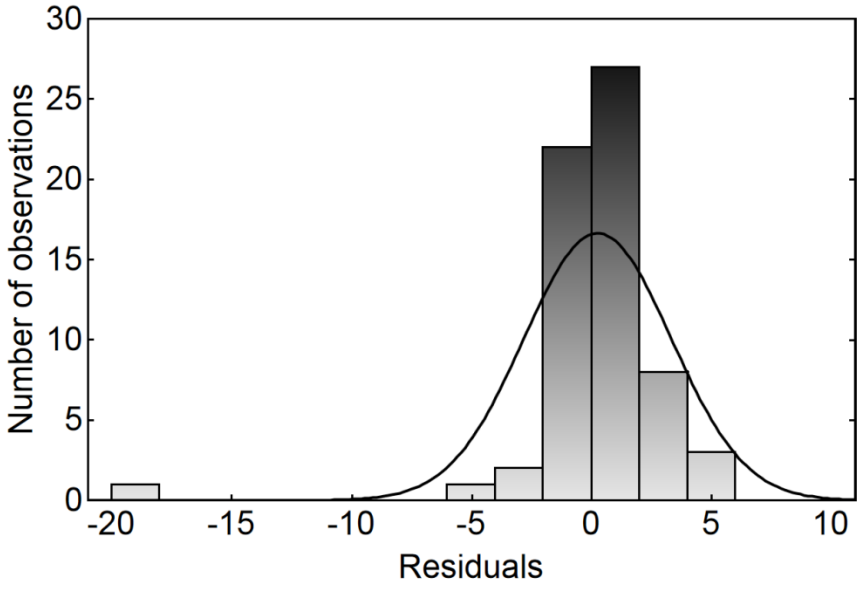

Fig 25. Normal distribution of residuals from a model on cyanobacterial biomass (Chi-square test 22.13, degrees of freedom 3, p 0.00).

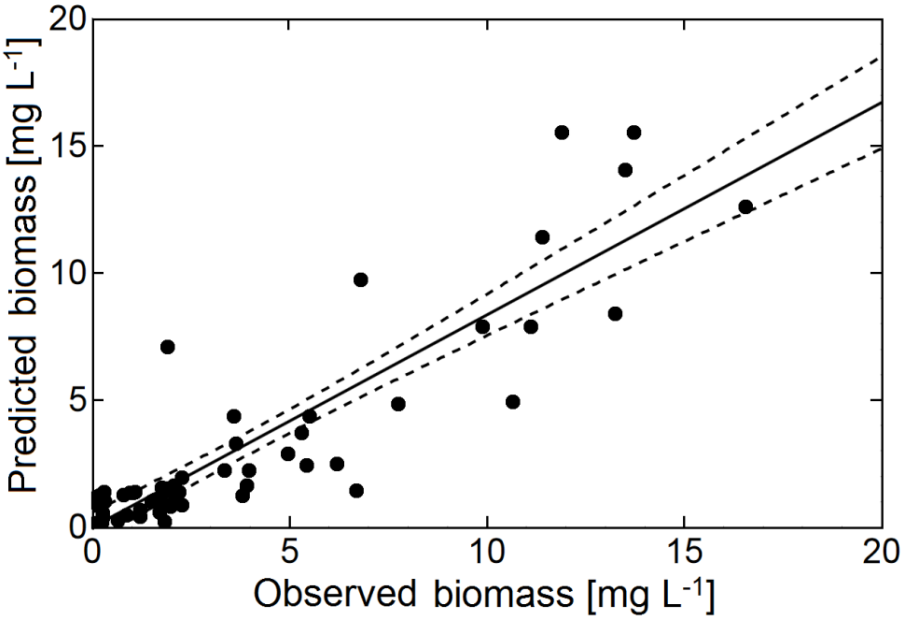

Fig 26. Goodness of fit of predicted values to observed cyanobacterial biomass values. Linear regression had a slope of 0.84. One outlier, which was produced by the model, is not shown in the fig (did not significantly change the slope).

Climatic influence

Significant changes in multiple variables were observed in 2010 in comparison to 2009 and 2011. It was found that these changes were most pronounced when the GML-system was divided into northern deep (dimictic) lakes (Przystań, Mamry, Dargin, Kisajno), southern deep lakes (Jagodne, Szymoneckie, Tałtowisko, Ryńskie, Tałty1, Tałty2, Mikołajskie and Bełdany) and polymictic lakes (Niegocin, Łabap, Boczne, Szymon and Śniardwy). Mean and standard deviation of multiple variables (concentrations of TN, TP, DOC and chl a, TN:TP ratio, number of heterocysts, biomasses of algae and cyanobacteria and thickness of OBZ) were calculated for each group of lakes in 2009, 2010 and 2011. An analysis of statistical significance of differences of the values in 2010 in comparison to 2009 and 2011 was done (fig 27).

Significant increases occurred in southern and northern deep lakes for chl *a*, DOC and TP concentrations in 2010 in comparison to 2009 and 2011. Additionally, the TN concentration and TN:TP ratio decreased significantly (fig 27). Cyanobacterial biomass was significantly higher in both southern and northern lakes, but the number of heterocysts was considerably higher only in the southern lakes. Biomass of algae increased in 2011 in the southern deep lakes and in the polymictic lakes, but no change was observable in the northern deep lakes. Changes in the depth of OBZ were not statistically significant.

In polymictic lakes, changes were generally more variable and in many cases insignificant. From further analyses, the majority of polymictic lakes were excluded, except Lake Niegocin. Reactions of Lake Niegocin were more similar to deep lakes than to other lakes with polymictic characteristics, possibly due to its strong stratification, especially in August 2010.

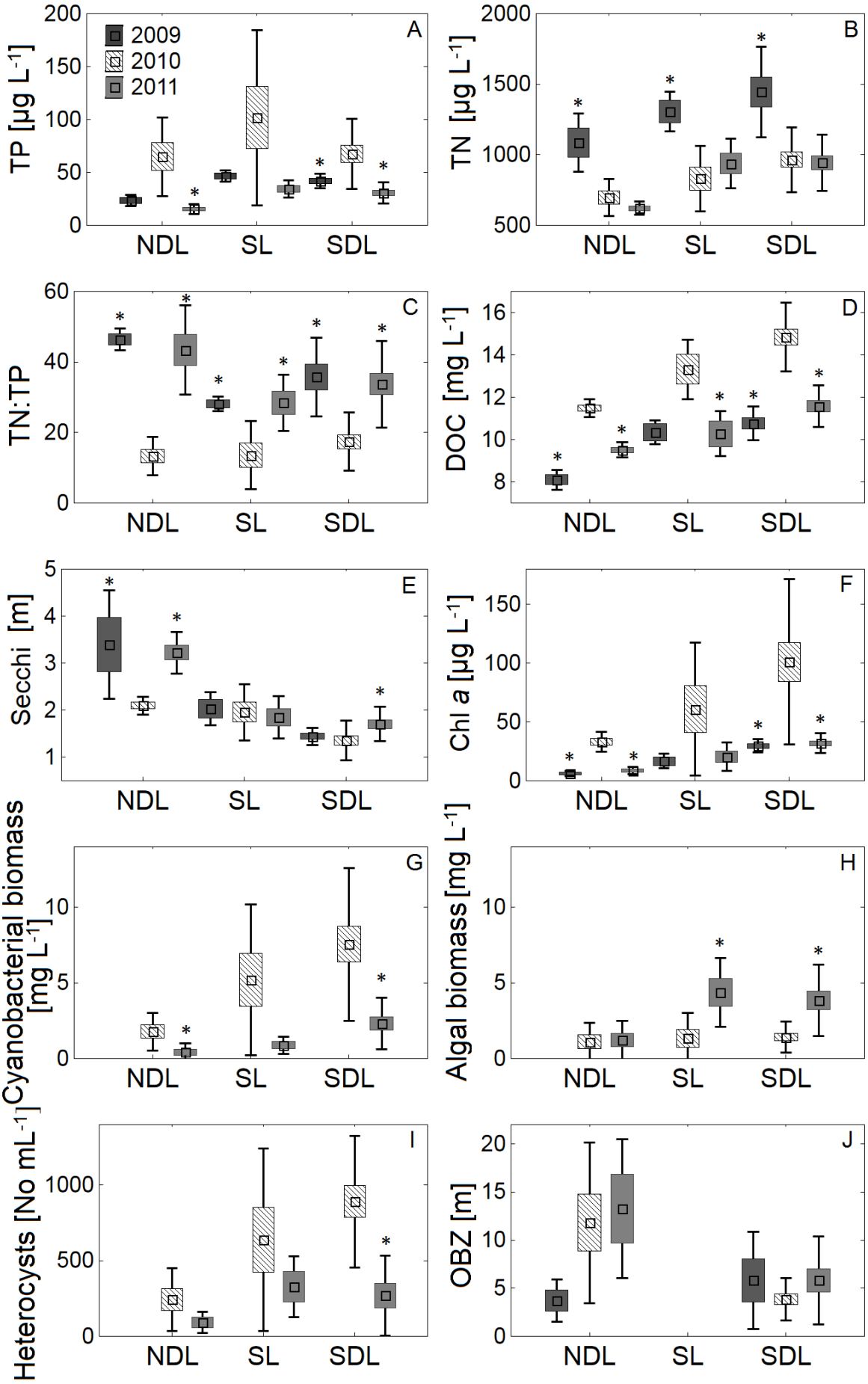
A
TP [µg L-1]
2009
2010
2011
NDL
SL
SDL
B
TN [µg L-1]
C
TN:TP
D
DOC [mg L-1]
E
Secchi [m]
F
Chl a [µg L-1]
G
Cyanobacterial biomass [mg L-1]
H
Algal biomass [mg L-1]
I
Heterocysts [No mL-1]
J
OBZ [m]

Fig 27. Differences in northern deep lakes (NDL), southern deep lakes (SDL) and "shallow" polymictic lakes (SL) in total phosphorus (A), total nitrogen (B), ratio of total nitrogen to total phosphorus (C), dissolved organic carbon (D), Secchi disk visibility (E), chlorophyll *a* (F), cyanobacterial biomass (G), algae biomass (H), number of heterocysts (I) and thickness of oxygenated buffer zone (J) between 2009, 2010 and 2011. Statistically significant differences between 2009 and 2011 in comparison to 2010 are indicated by a star above boxes. Mean - small squares, height of the box - standard error, thin lines - standard deviation.

Changes in water temperature and stratification structure of the lakes were analysed. Temperatures of epilimnia were significantly higher in 2010 than in 2009 and 2011. The mean epilimnion temperature of all dimictic lakes and Lake Niegocin was 19.6°C (SD 0.3) in 2009, 23.0°C (SD 1.6) in 2010 and 20.4°C (SD 0.9) in 2011 (fig 28). The highest recorded temperatures were found in lakes Tałtowisko (26.6°C), Ryńskie (26.5°C) and Tałty (site 1 25.8°C and site 2 25.1°C) in July 2010.

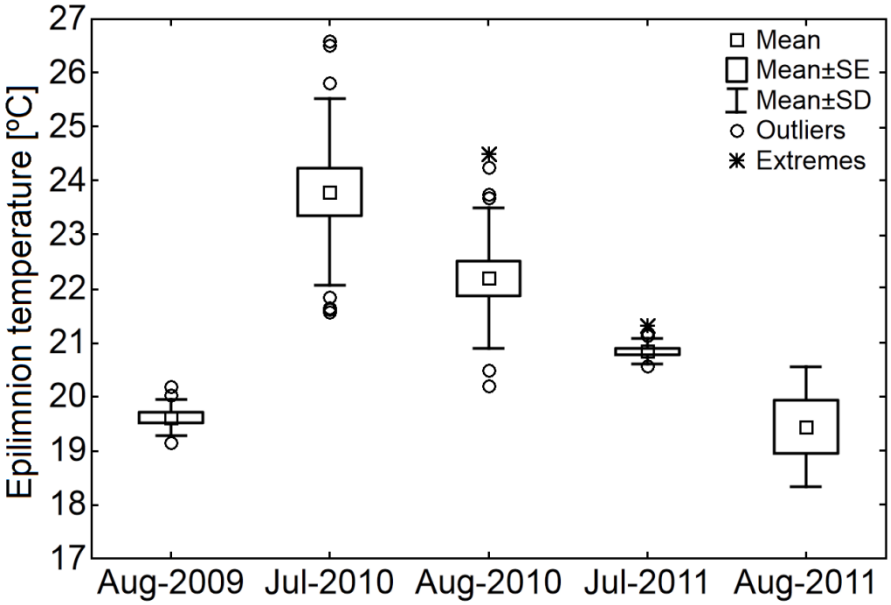

Fig 28. Temperature of epilimnia in the dimictic lakes of the GML-system and Lake Niegocin.

Consequences of the increased water temperature on stratification structure of the investigated lakes were analysed. This was done by comparing the epilimnion temperature to the epilimnion thickness (fig 29) and also by comparing the ratio of thickness of the euphotic water layer to the thickness of the epilimnion to water temperature (fig 30).

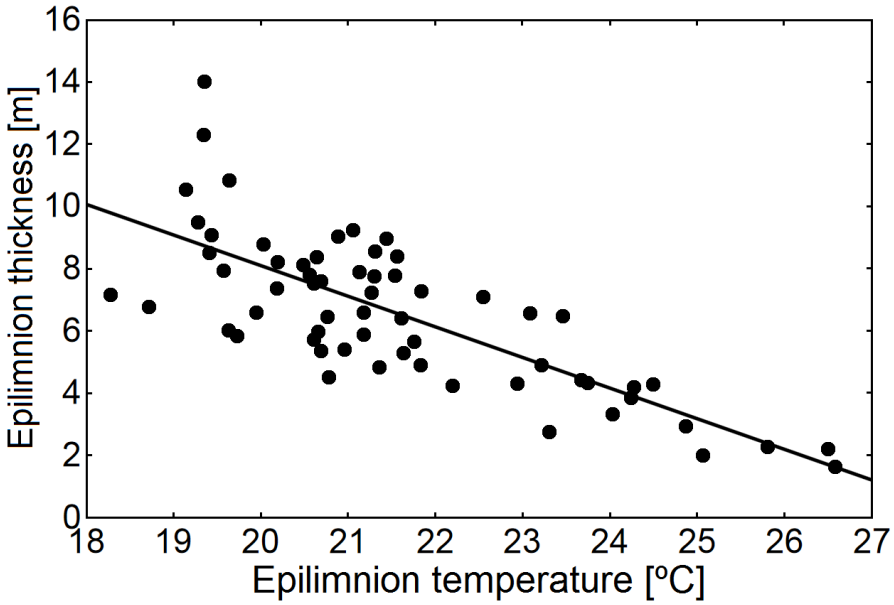

Fig 29. Correlation between thickness of epilimnion and water temperature (R^2 0.59).

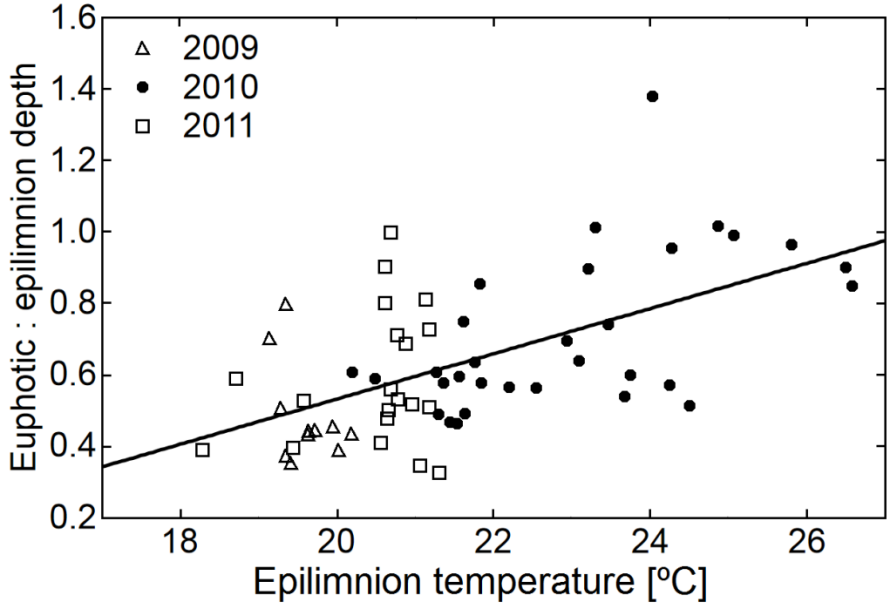

Fig 30. Ratio of thickness of euphotic zone to thickness of epilimnetic zone in comparison to water temperature (R^2 0.33).

The epilimnetic temperatures and thickness of epilimnia had a strong negative correlation (R^2 0.59, fig 29). The decrease in epilimnion thickness caused an increase in the ratio of the thickness of the euphotic zone to the epilimnetic zone in 2010 (fig 30). The ratio had mean values of 0.49 and 0.59 in 2009 and 2011, respectively; this ratio increased to 0.72 in 2010. In lakes Przystań, Tałtowisko, Ryńskie, Tałty (sites 1 and 2), Mikołajskie and Bełdany, the ratio

was closer to 1 than in other lakes (a range from 0.85 to 1.4 in July 2010, mean 1.0). No significant correlation of this ratio to chl *a*, biomasses of algae or cyanobacteria was found.

The proportion of the hypoxic water column from the maximum depth of a lake at the sampling site was analysed based on the oxygen saturation profile obtained with the YSI 6600-meter. The results obtained from 2010 were compared to results from 2009 and 2011 (fig 31).

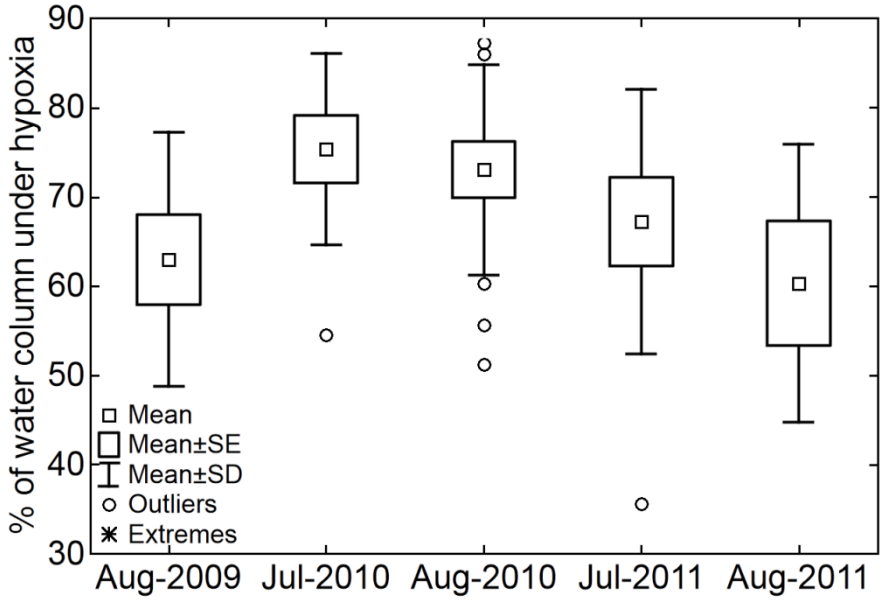

Fig 31. Percentage of water column under hypoxia during 2009-2011 (only lakes where hypoxia developed were analysed).

Changes in the thickness of the hypoxic water layer, expressed as a percentage of hypoxic water layer compared to the depth of the lake, were not statistically significant between years, due to high variance between lakes (fig 31). Still, a slight increase in the percentage could be observed in 2010.

The importance of hypoxia for the changes in 2010 was also investigated through a different approach. A distance between the upper border of the hypoxic water layer and the lower border of the epilimnion was calculated. This is the zone between the epilimnion and the hypoxic water layer, where oxycline exists. This zone is here termed the oxygenated buffer zone

(OBZ). Changes in the thickness of OBZ were analysed and compared to changes in limnetic variables (figs 32 and 33).

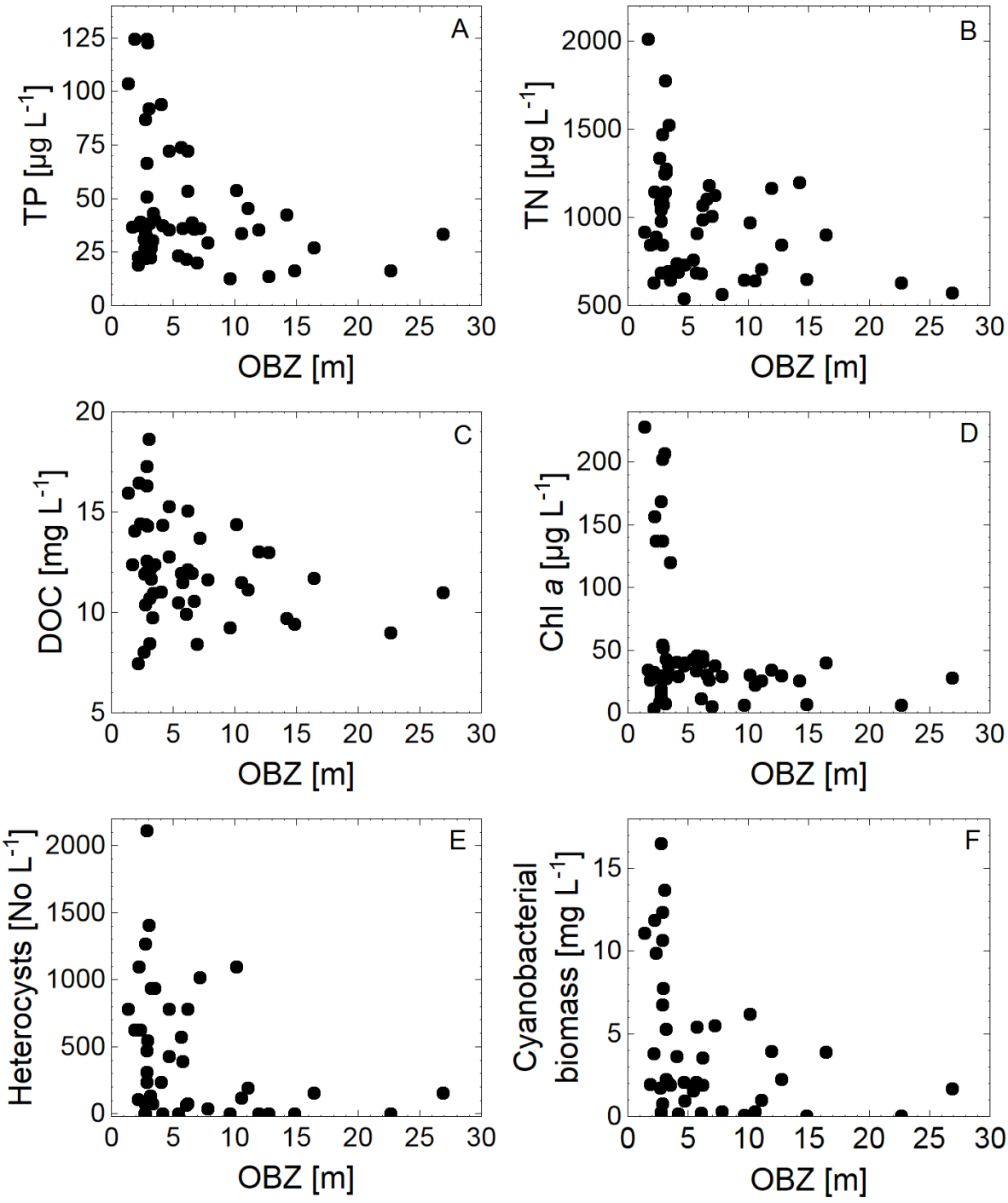

Fig 32. Plotted results of thickness of oxygenated buffer zone (OBZ) and concentrations of limnetic total phosphorus (TP, A), total nitrogen (TN, B), dissolved organic carbon (DOC, C) and chlorophyll *a* (chl *a*, D), number of heterocysts (E) and biomass of cyanobacteria (F) obtained from the GML-system in 2009-2011.

The thickness of OBZ was generally thinnest in 2010, but in some cases it was also thin during other years. However, when comparing the thickness of OBZ in 2010 to that in 2009 and 2011, the differences were not statistically significant. The thinnest OBZ was measured in Tałty 2 in August 2010, when it was just 1.3 metres thick. Generally, in lakes where hypoxia had developed, its thickness ranged from about 2 to 16 metres.

When the thickness of OBZ decreased below 5 metres, high increases in especially TP and chl *a* concentrations, the number of heterocysts and the biomass of cyanobacteria occurred (fig 32 A, D, E, F). A moderate increase in DOC was also observable, but this was much more gradual (fig 32 C). In contrast, the response of TN concentrations to the thickness of OBZ was more variable (fig 32 B). The TN concentration increased when the thickness was less than 5 metres only in 2009 and 2011, but not in 2010 (results not shown). In comparison, increases in TP concentrations mainly occurred in 2010. To analyse the significance of these changes, the variables were divided into two categories based on the thickness of OBZ (less than or more than 5 metres). Welch's t-test showed significant differences in concentrations of TP, TN, DOC and chl *a*, cyanobacterial biomass and number of heterocysts between the two categories.

It was also attempted to analyse how limnetic and profundal TP concentrations and the thickness of OBZ influenced each other. A three-dimensional surface plot with limnetic and profundal TP concentrations and the thickness of OBZ was constructed using a negative exponential smoothing procedure (fig 33). However, profundal TP concentrations were only measured in 2009 and 2010. The profundal TP concentration did not show significant variability between years and the differences between lakes were much larger. Based on these factors and for the purposes of visual demonstration only, profundal TP concentration in each deep lake in 2011 was evaluated as a mean of concentrations from 2009 and 2010. This did not significantly alter the shape of the three-dimensional surface plot, but made the results more easily visible.

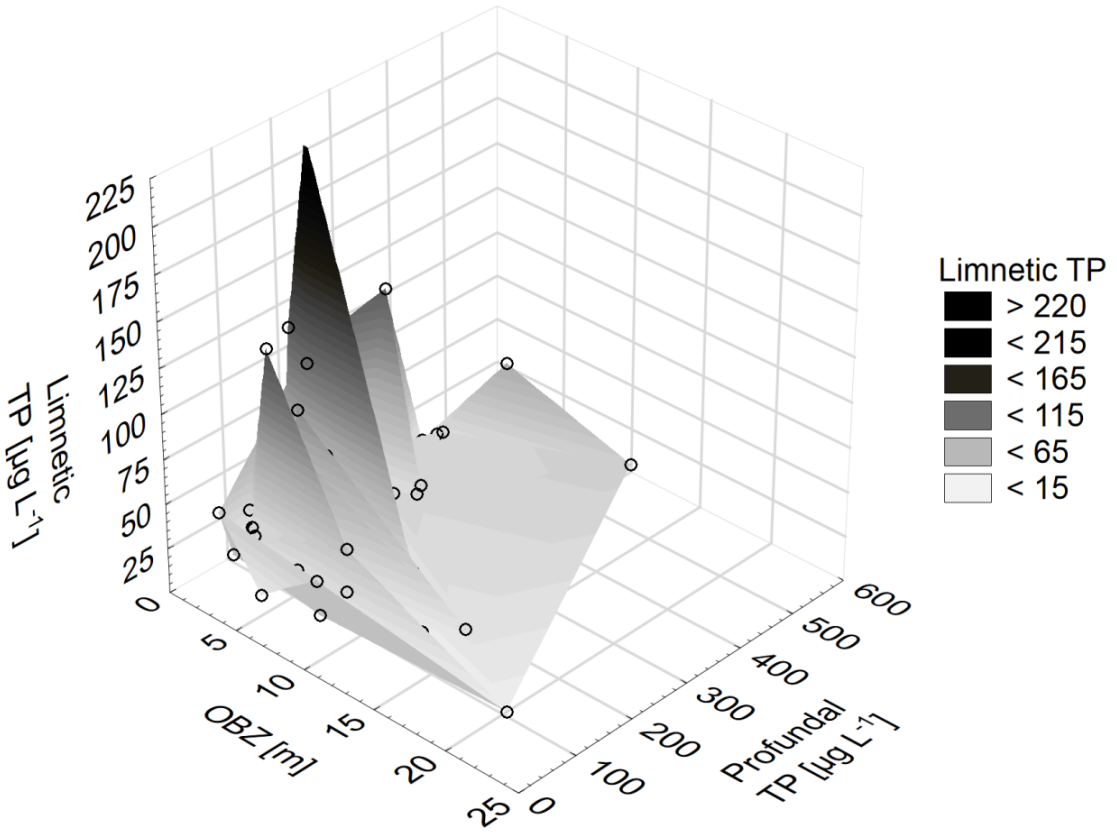

Fig 33. Increase in total phosphorus (TP) concentration in limnetic zone on a plane of changing thickness of the oxygenated buffer zone (OBZ) and profundal total phosphorus concentration. Negative exponential smoothing procedure was used on the surface plot. Raw data points are shown as circles. Profundal TP concentrations in 2011 were evaluated based on mean from values from 2009 and 2010.

The increases in limnetic TP concentrations occurred only in lakes where two factors occurred simultaneously: the OBZ was shallow and profundal TP concentrations were high (fig 33). All lakes where the limnetic TP concentration increased above 80 µg L^{-1} had profundal TP concentrations above 100 µg L^{-1} (on average 184 µg-P L^{-1}, SD 65) and OBZ shallower than 4.0 m (on average 2.8 m, SD 0.8). These lakes included Mamry, Łabap, Niegocin, Boczne, Jagodne, Szymoneckie, Tałtowisko and both parts of Tałty in August 2010.

Vertical entrainment in the GML-system was calculated based on temperature profile data from summer 2010 (figure 34). The rate of entrainment ranged between 1E-08 and 1E-06. In lakes Boczne, Szymoneckie and Bełdany entrainment was negative and in the rest of the lakes

positive. No relationship between rate of entrainment and epilimnetic nutrient concentrations was found.

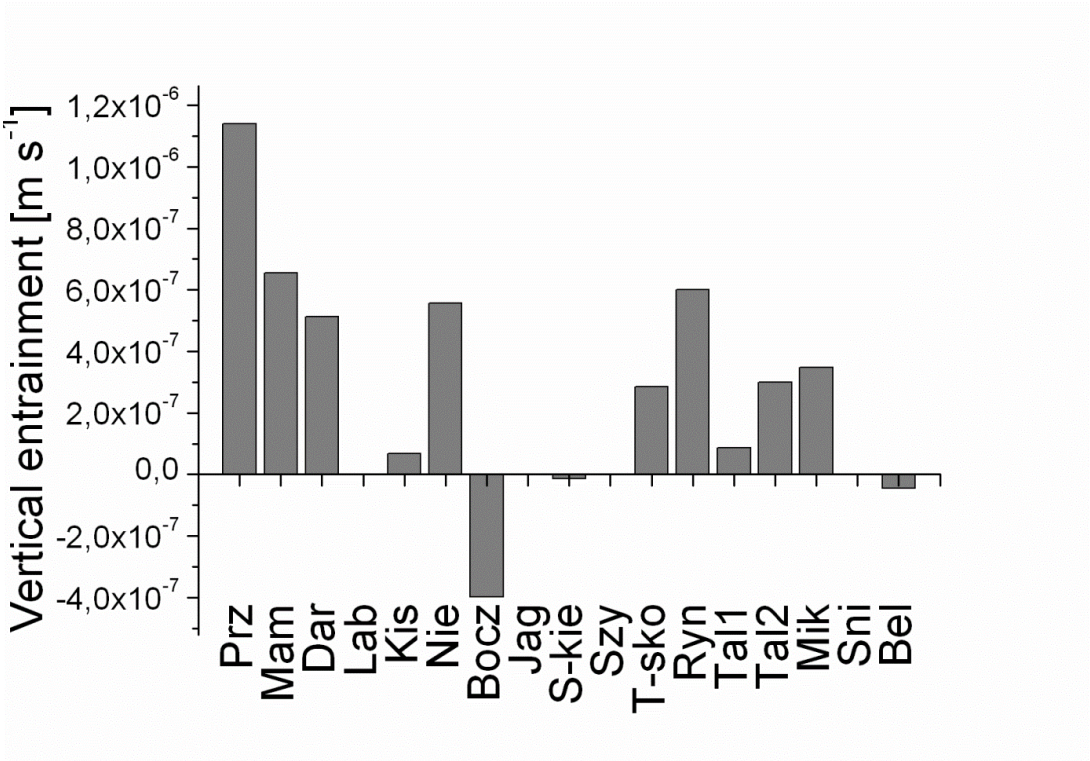

Figure 34. Vertical entrainment in the GML-system in year 2010.

Sensitivity to further oligotrophication

In order to explain differences in TSI between lakes of the GML-system, their limnological characteristics, which are known to influence functioning and trophic status, were investigated. The characteristics were chosen using the criteria that they showed differences between lakes and that there were data available for each lake. Data about lake volume were acquired from Jańczak (1999). The variables land use, existence of vegetative riparian buffers, shape of the lake and connectivity of lakes were determined from available maps. Mean sulphate concentration for each lake was calculated as a mean value based on data acquired during summer months from 2009 to 2011.

The characteristics of each drainage area are presented in table 6. Land use was classified as agricultural, towns, forest or as a mix of multiple land uses. The presence of vegetative riparian buffers (defined as: an approximately 20 metre-wide strip of trees or bushes that separates agricultural land from lake water) was classified as full, partial or no coverage on the riparian buffers in areas where adjacent land was under agricultural usage.

Shape of a lake was characterised as circular, slightly elongated or elongated. Connectivity of a lake was determined based on inflows and outflows of water: if a lake had more than one natural inflow and outflow, connectivity was classified as "good"; if a lake lacked either an inflow or an outflow connectivity was classified as "partial"; and if a lake either did not have an inflow or an outflow, or only had artificial channels, the connectivity was classified as "bad".

The characteristics were classified and points were assigned to each lake according to tables 6 and 7. A sum of points based on these characteristics correlated positively with mean TSI-values from 2009-2011 (R^2 0.56, fig 35).

Table 6. Characteristics of drainage area of the GML-system. Points were assigned to each lake based on classes, as shown in table 7. Lake volume information from Jańczak (1999).

Lake	Land use in vicinity of lake	Riparian buffers	Significant point-sources (towns)	Shape of lake	Lake volume [hm^3]	Connectivity	SO_4 [mg L^{-1}]	Sum of points
Przystań	Agricultural-forest	No		Round	298	Good	9	5
Mamry	Agricultural-forest	Full		Round		Good	8	2
Dargin	Agricultural-forest, settlements	Full		Round	322	Good	12	3
Łabap	Agricultural	Full		Round		Good	11	4
Kisajno	Agricultural-forest, settlements	Full		Slightly elongated	159	Good	9	4
Niegocin	Agricultural-forest, settlements	Partial	Town Giżycko	Round	259	Partial	17	8
Boczne	Agricultural-forest, settlements	No		Slightly elongated	16	Good	14	7
Jagodne	Agricultural-forest, settlements	No		Slightly elongated	83	Good	13	7
Szymoneckie	Agricultural, settlements	No		Slightly elongated		good	16	8
Szymon	Agricultural	Partial		Round	2	Bad	13	8
Tałtowisko	Agricultural-forest	Partial		Elongated	46	Bad	14	10
Ryńskie	Agricultural-forest, settlements	Partial	Town Ryn	Elongated	67	Partial	14	10
Tałty I	Agricultural-forest, settlements	Partial		Elongated	181	Good	15	8
Tałty II	Agricultural, settlements	No	Sewage input from Mikołajki	Elongated		Good	14	10
Mikołajskie	Agricultural-forest, settlements	Partial	Town Mikołajki	Elongated	56	Good	13	9
Śniardwy	Agricultural-forest, settlements	Partial		Round	660	Good	9	3
Bełdany	Forest-agricultural, settlements	Full		Elongated	95	Good	7	3

Table 7. Appointment of points for drainage and basin area influence.

Points	Land use in vicinity of lake	Riparian buffers	Significant point-sources (towns)	Shape of lake	Lake volume	Connectivity	SO_4, sulphate
0	Forest	Full	none	Round	200-	Good	> 10 mg L^{-1}
1	Agricultural-forest	Partial		Slightly elongated	50-200	Partial	10-15 mg L^{-1}
2	Agricultural, agricultural-forest with settlements	No	Towns or sewage input from a town	Elongated	0-50	Bad	< 15 mg L^{-1}

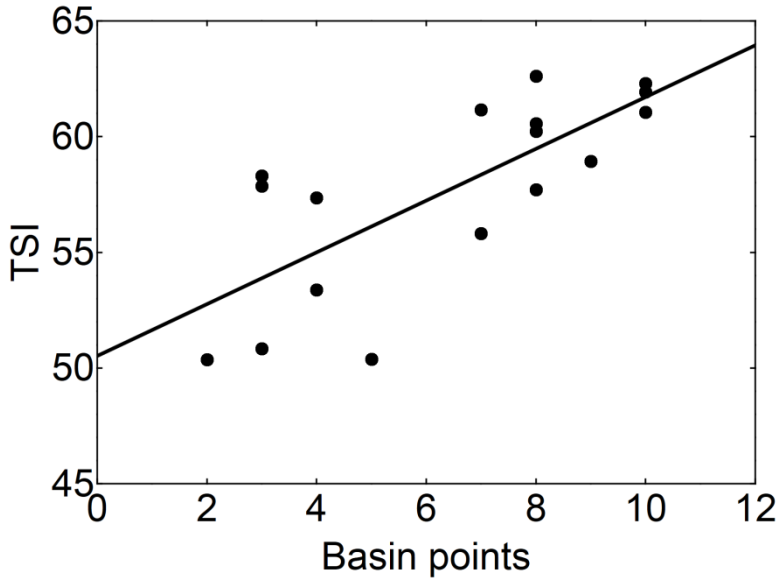

Fig 35. Correlation between points based on characteristics of drainage areas and mean TSI based on data collected during summer seasons 2009-2011 (TSI = 50.3 + 1.1**Basin points*, R^2 0.56).

Factors influencing sulphate concentration in the limnetic zone of the GML-system were investigated. Sulphate concentrations were compared to TSI and the existence of vegetative riparian buffers. It was found that sulphate concentrations had a strong positive correlation with TSI (R^2 0.32, results not shown). An increase in sulphate concentration was observed when vegetative riparian buffers did not exist or only partially covered the shoreline where agricultural fields were directly adjacent to lakes (fig 36).

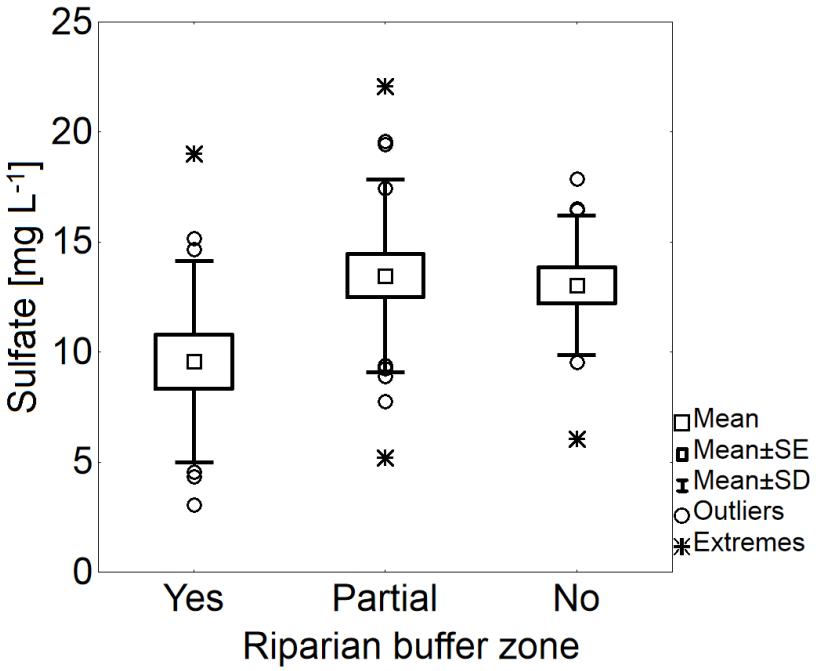

Fig 36. Sulphate concentration [mg L^{-1}] in lakes with full, partial or absence of vegetative riparian buffers.

DISCUSSION

Intensive eutrophication phase during 1960-1990

Historical information about the trophic state of the GML-system was gathered from the available literature. Data concerning the years before 1990 was sparse; thus, it will only be reviewed here shortly.

According to Lawacz et al. (1978), human influence in the Great Masurian Lakes area began during the 15th century with human settlement, deforestation and agricultural development, leading to a slow rise in productivity (Gliwicz and Kowalczewski 1981). Cohn found water transparencies ranging from 3 metres (Lake Kisajno) to 6.5 metres in 1903 (Lake Niegocin), meaning that at least some of the lakes at that point were oligotrophic (Gieysztor and Odechowska 1958). Until the 1960s, Lake Mikołajskie had Secchi disk visibility of 3.0 metres (Gliwicz 1969).

Intensive eutrophication of lakes began slowly with the intensification of agriculture and bad agricultural practices (such as inappropriate ways of storing manure, lack of vegetative riparian buffers around lakes and over-fertilisation) around the 1950s. Other anthropogenic sources of nutrients were the development of tourism and the expansion of villages and towns. During the 1970s, half of the lakes received domestic sewage directly from municipal or camping areas (Ozimek 1975); municipal sewage accounted for as much as 86% of total phosphorus run-off into Lake Mikołajskie (Górski and Rybak 1974). In the GML-system, direct point sources of phosphorus made up 42% of all phosphorus inputs, while inputs from other tributaries were almost equal and made up 46% of total phosphorus inputs in 1976 (Giercuskiewicz-Bajtlik and Głąbski 1981). The pollution load from sewage to the whole GML-system was estimated to be 127 thousand kg-N year^{-1} and 32 thousand kg-P year^{-1}. The rest of

the inputs (12.5%) originated from diffuse sources. The high pollution load led to intensive eutrophication. This was observable, for example, as a low Secchi disk visibility—in Lake Mikołajskie, the average Secchi disk visibility was 1 metre during the 1970s (Kajak et al. 1975).

During the eutrophication phase of the GML-system, vegetation and plankton also went through strong changes, reflecting the high increase in trophic state. It was observable as a change in vegetation type from *Chara*-type through *Potamogeton*-type, and eventually to *Myriophyllum-Potamogeton*-type (Kowalczewski and Ozimek 1993). At the same time, an increase in filamentous algae growing on macrophytes (Pieczyńska et al. 1988) and a general decrease in water transparency led to a decrease in submerged macrophyte cover (in Lake Mikołajskie, the area decreased from 48 ha in 1963 to about 28 ha in 1990; Kowalczewski and Ozimek 1993, Ozimek and Kowalczewski 1984). Furthermore, collapses in populations of macrophytes, which are strong competitors for phosphorus (e.g. *Chara*), may have further benefited algae and cyanobacteria (Kufel and Ozimek 1994). Additional collapses in vegetation may have caused decreases in different species populations, which depend on the vegetation. For example, zebra mussel (*Dreissena polymorpha*) populations rely on suitable macrophyte substrate (especially *Chara*) for protection from predators (Stańczykowska and Lewandowski 1993).

Eutrophication can also cause changes in the composition of fish community. While populations of cyprinids usually expand, the biomass of piscivore species such as pike (*Esox lucius*) decreases. According to Wołos et al. (2009), commercial fish catches from Lake Mamry were at their highest during the 1960s and early 1970s and have been decreasing since. Pike catches collapsed at the end of the 1970s. The authors concluded that the development of fish catches (with decrease in pike and perch, *Perca fluviatilis*, catches and increase in cyprinid species) fit well with the general trend of eutrophication.

The fastest rate of eutrophication of the southern lakes occurred between 1970 and 1990, and they became hypereutrophic during the 1980s (mean TSI of all southern lakes was 72 according to database of Microbial Ecology Department). The northern lakes of the GML-system did not go through a strong eutrophication phase; they have steadily maintained mesotrophic status with some temporary eutrophic characteristics since the 1970s (Wołos et al. 2009, Zdanowski et al. 2009). There is a possibility that positioning of the drainage divide between northern and southern lakes has changed (Bajkiewicz-Grabowska 2008), which could have influenced the direction of flow from a sewage treatment plant in Giżycko. Usually, nutrients from sewage flow from lake Niegocin towards Boczne and Jagodne (southwards), but a change in the drainage divide could have caused a nutrient rich water flow towards the northern lakes instead (Bajkiewicz-Grabowska 2008). Usually, the main sources of nutrients in the northern lakes of the GML-system are diffuse inputs such as tourism, agriculture and atmospheric deposition (Wołos et al. 2009). The atmospheric deposition accounted for about one fourth of all diffuse sources of phosphorus into Lake Mamry (887 kg-P $year^{-1}$ to direct drainage area of Mamry).

The state of the GML-system from 1960 to 1990 serves as a baseline for the following ecological changes, which are the main focus of this thesis. More information about the period of intensive eutrophication can be found in Siuda et al. (2013).

Oligotrophication and current state of the GML-system

Nutrient inputs to the southern lakes of the GML-system began to decrease during 1990-1995 (will be discussed in the next chapter) and the first signs of oligotrophication of the southern lakes appeared (fig 2). Since then, the trophic state has been improving and has stabilised at eutrophic class (mean TSI 59) since 2000. TP concentrations decreased to one fourth of the former concentrations within about 10-15 years (mean from 1980-1989 was 206 µg L^{-1}

and from 2000-2011 51 µg L^{-1}). The limnetic TP concentrations reached a new stable state after the decrease in external inputs fast, but within a typical time frame found in similar cases (Jeppesen et al. 2005). In addition, Secchi disk visibility improved from an average of 0.9 metres during 1990-1999 to 1.5 metres during 2000-2011. In contrast, no significant improvement was observable in chl *a* concentrations (mean concentration was 38 µg L^{-1} during 1990-1999 and 37 µg L^{-1} during 2000-2011).

The northern lakes of the GML-system did not have a clear long-term trend in the development of trophic state, due to lack of consistent data before 1998 and the low frequency of published measurements. They do not seem to have experienced intensive eutrophication at any point and have mostly remained as meso-eutrophic (fig 2). For example, mean TSI was 46 during the 1980s, 52 during the 1990s and 49 during the years 2000-2011. Also, neither chl *a* concentration nor Secchi disk visibility changed significantly with time. Only TP concentrations showed a decrease from 52 µg L^{-1} during the 1990s to 34 µg L^{-1} during 2000-2011.

One of the most polluted lakes in Poland was Lake Niegocin, which went through a very conspicuous oligotrophication; the lake was hypereutrophic during the 1980s (mean limnetic TP concentration in was 435 µg L^{-1} and TSI 81). Installation of chemical phosphorus precipitation technology to the wastewater treatment plant in town Giżycko in 1994 led to fast improvements in the ecological state of the lake. The trophic state began to decrease immediately at a fast rate (mean TP concentration was 132 µg L^{-1} and TSI 68 during years 1994-2000, fig 3). Since then, the improvements have been slowly continuing (mean TP concentration was 80 µg L^{-1} and TSI 60 during 2001-2011). Thanks to the decreased TP concentration, cyanobacterial biomass also declined to a point where barely any cyanobacteria were observable in 1996 (Narpiórkowska-Krzebietke and Hutorowicz 2006). However, high cyanobacterial dominance returned by the year 1999. According to the database of the Microbial Ecology Department and data gathered during the present investigations, chl *a* concentrations did not show a similar

reduction and have actually been increasing since 2000. These results suggest that cyanobacterial biomass in Lake Niegocin continues to go through large variations. For example, about 43% of phytoplankton biomass were cyanobacteria in 2010, but the proportion decreased to just 5% in 2011. For some reason, phytoplankton and especially cyanobacteria are not responding to decreases in external nutrient inputs and in limnetic TP concentrations in the expected manner.

Lake Mikołajskie went through similar distinct oligotrophication as Lake Niegocin. Limnetic TP concentrations decreased steadily from the 1980s to one fourth of the previous values (mean TP was 175 µg L^{-1} in 1981-1990 and 43 µg L^{-1} in 2001-2011); however, there was no observable trend in TP concentration after 2000, indicating that the decrease had stopped. Also chl *a* concentrations have remained in the same range since 1990 (mean 32 µg L^{-1}), except for 2010 when a high increase was observed (in August 137 µg L^{-1}). The stop in reduction of TP and chl *a* concentrations was unexpected because external inputs decreased according to BDL (2012) in 2006. The decrease was achieved by upgrading the municipal wastewater treatment plant. This might indicate that limnetic TP and chl *a* concentrations have become dependent on factors other than external point sources.

Oligotrophication of Lake Ryńskie was less dramatic than in lakes Niegocin and Mikołajskie, but was still clearly observable. Phosphorus concentrations decreased to about half of the mean values of the 1980s (from 79 µg-P L^{-1} during 1980-1989 to 43 µg-P L^{-1} during 1995-2005). However, since 2005, the trophic state of Lake Ryńskie has slightly increased. Similarly to lakes Niegocin and Mikołajskie, chl *a* concentrations in Lake Ryńskie did not respond clearly to oligotrophication (mean was 37 µg L^{-1} from years 1990-1999 and 36 µg L^{-1} from 2001-2011). A high increase in chl *a* concentrations in 2010 was also observed in Lake Ryńskie (207 µg L^{-1} in August 2010). These trends did not follow the variations in nutrient load from the wastewater treatment plant in Ryn (BDL 2012), indicating that the TP and chl *a* concentrations also depend on some other factor than external point sources.

The southern and northern lakes of the GML-system have had a very different development of trophic state. Previously, there were large trophic state differences between the two parts of the GML-system (lakes ranged from oligo-mesotrophy to hypereutrophy according to Chróst and Siuda 2006, for example), but the trophic state differences have almost disappeared during recent years. The mean TSI of all lakes of the GML-system ranged between 50 and 63 during 2009-2011 (eutrophic class), but went through quite large variations between years (fig 7). In fact, variation within a lake was often larger than the differences between lakes within one year. Still, the northern lakes generally remained at a lower trophic state than the southern lakes. Using mean TSI based on data only from 2009 and 2011, lakes Przystań, Mamry and Dargin could have been classified as mesotrophic, and the rest as eutrophic. Also, the phytoplankton biomass and activity of organisms (enzymatic activity, rates of primary production and respiration) were generally lower in northern lakes than in the southern lakes.

Oligotrophication of lakes Niegocin and Boczne has progressed to such extent that their mean TSI, phytoplankton biomass and activity of organisms were typically in between mean values encountered in the northern lakes and in other southern lakes (figs 2, 13, 18) during 2009-2011. For example, the mean TSI was 58 in Niegocin and 56 in Boczne, in comparison to 53 in the northern lakes and 61 in other southern lakes. The average of total phytoplankton biomass was 3.5 mg L^{-1} in Niegocin and 3.2 mg L^{-1} in Boczne, in comparison to 2.5 mg L^{-1} in the northern lakes and 8.0 mg L^{-1} in the southern lakes.

The lack of clear trend in chl *a* concentrations and Secchi disk visibility in the GML-system was partly due to the lack of data from the 1980s and early 1990s. However, it has been observed by many researchers that phytoplankton biomass, chl *a* concentrations and Secchi disk visibility are not as responsive to decreases in external inputs as TN and TP concentrations are (Dokulil and Teubner 2005, Jeppesen et al. 2005). Their reaction often has a delay which lasts for multiple years. This seems to also be the case in the GML-system. In the southern lakes, a

decrease in limnetic TP concentrations was followed by a decrease in chl *a* concentrations only after a delay, which lasted for about 5-8 years (fig 4). However, chl *a* concentrations began to increase again after 2000, without a similar trend being observed in limnetic TP concentrations.

The situation where chl *a* concentrations have failed to respond properly to a decrease in limnetic TP concentrations may have multiple explanations. One of the possibilities is internal loading of phosphorus. When phosphorus is released under low redox-conditions from sediments, it is in its inorganic form and can be easily assimilated by phytoplankton. Thus, its "capacity" to form new phytoplankton biomass could be expected to be greater than that assumed based on TP concentration. This could be one of the reasons why log-transformed chl *a* and TP concentrations measured in the GML-system are often above Dillon and Rigler's ratio (1974, fig 22). However, there are no direct measurements of internal loading of phosphorus in the GML-system, but there are some indirect indications about its occurrence. Internal loading of phosphorus could occur during lake overturns when the water column is thoroughly mixed. In the southern lakes of the GML-system, increased limnetic TP concentrations were observed regularly during spring overturn (figs 5 and 6). It is possible that phosphorus rich sediments become resuspended in the water column during the overturns. The occurrence of internal loading of phosphorus in the GML-system is also supported by the fact that profundal hypoxia remained a persistent phenomenon during summer months in multiple southern lakes (fig 8). Only lakes Szymon and Łabap never showed signs of hypoxia, while other lakes (Niegocin, Boczne, Śniardwy and northern lakes) developed hypoxia with irregular patterns. High profundal TP concentrations were measured during the summer in many of the lakes where hypoxia was common (lakes Jagodne, Szymoneckie, Ryńskie, Tałty site 2, Mikołajskie and Bełdany, fig 9). When both profundal hypoxia and high TP concentrations occur together, internal loading of phosphorus could become a possibility. In addition to hypoxia possibly causing internal loading of phosphorus, it can also cause other problems in aquatic ecosystems (fish deaths, decreased

amount of available oxygenated habitat, etc.). Another possible explanation for unresponsiveness of phytoplankton to oligotrophication could be an unfavourable trophic structure, where planktivores are dominating (Carpenter et al. 1992, Jeppesen et al. 2002); however, there are no available data to investigate this possibility.

Generally, the number of heterocysts, and biomasses of algae and cyanobacteria varied greatly between 2010 and 2011 (figs 10 and 11). It is known that phytoplankton communities can have a variable and almost chaotic response to oligotrophication (Dokulil and Teubner 2005). However, it appears that the large changes in chl *a* levels and the phytoplankton community during 2009-2011 were not only a response to oligotrophication but were influenced by other variables (discussed later).

Development of external nutrient inputs

The scientific opinion that phosphorus is the main cause of eutrophication in freshwater ecosystems has mainly been prevailing since the 1970s (Schindler 2006, 2012, Smith et al. 2006). Because of that, eutrophication of freshwater ecosystems has been controlled mainly through the regulation of external phosphorus inputs. Globally, one of the first targets of action was phosphorus in laundry detergents (sodium tri-polyphosphate). Usage of phosphorus in dishwashing detergents was not banned in Poland, but phosphorus content has been restricted to 6% since 1995 (before 1991 the allowed level was 9%). A complete ban of phosphorus-based detergents could achieve a 10-40% (EU Commission 2007) or even 25-50% (Metzner 2001) decrease in the phosphorus load entering surface waters in comparison to a situation where only phosphorus-based detergents are in use.

External nutrient inputs in the region of the GML-system have followed similar developments as in Poland in general. Improvements of wastewater treatment facilities, and decreases in agriculture and fertiliser usage all began around 1990. In the Warmia-Masuria

province, the percentage of the population connected to wastewater treatment plants increased from 65% in year 2000 to 72% in 2010 (USO 2011a). Simultaneously, the proportion of the population connected to wastewater treatment with improved nutrient removal increased from 32% in 2000 to 57% in 2010. Discharge of untreated wastewaters to the GML-system is relatively small in comparison to the national average: in 2010, only 0.4% of all wastewaters requiring treatment were released without treatment; nationally, the value was 7.6% (GUS 2011, USO 2011b).

The main sources of nutrients in the GML-system are the towns Giżycko (population about 29000), Ryn (population about 3000) and Mikołajki (population about 3700). Wastewater treatment plants in Giżycko and Mikołajki are now equipped with improved nutrient removal systems, but the plant in Ryn is still only based on biological treatment (BDL 2012). Wastewater treatment in Giżycko has gone through multiple changes. First wastewater treatment facility in Giżycko was built in the 1960's and had only mechanical treatment. A new plant was built in 1984, but still no decrease in the degradation of the water in Lake Niegocin was observable. This can be seen in the trophic state index during the 1980s: Lake Niegocin persistently maintained a hypereutrophic status for years (the mean TSI in 1980s was 86, in comparison to 75 in Mikołajskie and 67 in Ryńskie). Since the wastewater treatment plant in Giżycko was updated in 1994 with improved nutrient removal system, the nutrient load has significantly decreased, which has allowed Lake Niegocin to recover. The final round of upgrades was completed in 2002, which enabled the plant to adhere to the highest standards in Poland and in EU. These improvements may not have had a significant effect on the amount of phosphorus being discharged: the mean from 1998-2002 was 1205 kg-P $year^{-1}$ and during 2003-2011 was 1410 kg-P $year^{-1}$ (BDL 2012). However, the amount of nitrogen in discharged wastewater seems to have decreased strongly: the mean from 1998-2002 was 26350 kg-N $year^{-1}$ and from 2003-2011 was 19070 kg-N $year^{-1}$ (table 3).

The wastewater treatment plant in Mikołajki was built in the 1990s and its capacity was increased in 2006 from 1550 m^3 day^{-1} to 3900 m^3 day^{-1} (BDL 2012). Simultaneously, wastewaters from new areas were directed to the plant and the nutrient removal efficiency was improved. Thus, according to data made available by BDL (2012), the total nutrient load from the plant decreased strongly: the means from 1998-2006 were 327 kg-P $year^{-1}$ and 6202 kg-N $year^{-1}$ and from 2007-2011 were 219 kg-P $year^{-1}$ and 5039 kg-N $year^{-1}$.

The capacity of the wastewater treatment plant in Ryn was upgraded in 2006 from 1000 to 1300 m^3 per day (BDL 2012). The nutrient load discharged to Lake Ryńskie goes through large yearly variations; the minimum phosphorus load was recorded by BDL in 2009, when only 68 kg-P was discharged in a year. In contrast, in 2000, 1670 kg-P was released. Mean pollutant loads calculated based on data from BDL (2012) from 2009-2011 were: 911 kg-P $year^{-1}$ (SD 3778) and 5491 kg-N $year^{-1}$ (SD 483). However, when contacting officials in Ryn, they reported that the nutrient load from the wastewater treatment plant was 256 kg-P $year^{-1}$ and 3066 kg-N $year^{-1}$ on average. However, these values are probably theoretical means when the plant is not experiencing problems (i.e. too much rain causing overflow, etc.). Data published by BDL are based on early or half-yearly reports which the authorities of the treatment plants are legally obliged to provide and are more likely to be correct.

The towns of Ryn and Mikołajki are relatively equal in size, but due to the improved nutrient removal in Mikołajki's wastewater treatment plant, it releases only about 176 kg-P $year^{-1}$ (mean from years 2009-2011) while Ryn's plant releases about 805 kg-P $year^{-1}$. The total nutrient load from the wastewater treatment plant to Lake Ryńskie is similar to the load to Lake Niegocin, despite large differences in the populations of the towns.

Assuming an even distribution of the pollutant load from the treatment plants to the whole area of receiving lake, the highest phosphorus load during 2009-2011 was in Lake Ryńskie (about 119 mg-P m^{-2} $year^{-1}$), or in Ryńskie-Tałty as a whole (44 mg-P m^{-2} $year^{-1}$, table

4). Lakes Niegocin and Mikołajskie have lower phosphorus loads (37 mg-P m^{-2} $year^{-1}$ and 35 mg-P m^{-2} $year^{-1}$, respectively). In comparison, Giercuszkiewicz-Bajtlik and Głąbski (1981) estimated that total phosphorus load from point sources (does not include inputs from rivers or other lakes) in 1976 was 60 mg-P m^{-2} $year^{-1}$ in lake Ryńskie-Tałty, 970 mg-P m^{-2} $year^{-1}$ in Niegocin and 670 mg-P m^{-2} $year^{-1}$ in Mikołajskie. These values show 96% and 95% decreases from the 1970s to the present day in the load of phosphorus from wastewater treatment plants to lakes Niegocin and Mikołajskie, respectively, but only a 27% decrease in load to lake Ryńskie-Tałty.

The pollution load of nitrogen from wastewater treatment plants followed a different pattern: nitrogen load during 2009-2011 was the highest in Lake Mikołajskie (1095 mg-N m^{-2} $year^{-1}$), while lakes Niegocin, Ryńskie and Ryńskie-Tałty had lower nitrogen loads (771 mg-N m^{-2} $year^{-1}$ and 363 mg-N m^{-2} $year^{-1}$ or 134 mg-N m^{-2} $year^{-1}$, respectively). In comparison to data presented by Giercuszkiewicz-Bajtlik and Głąbski (1981), the reductions in nitrogen loading were much smaller in Mikołajskie and Niegocin than the reductions in phosphorus loading. Estimated point source nitrogen loads according to Giercuszkiewicz-Bajtlik and Głąbski in 1976 were 2760 mg-N m^{-2} $year^{-1}$ in Lake Mikołajskie, 3600 mg-N m^{-2} $year^{-1}$ in Niegocin and 640 mg-N m^{-2} $year^{-1}$ in Ryńskie-Tałty. The reductions in comparison to present wastewater inputs were 60%, 79% and 79%, respectively. In Ryńskie-Tałty, external nitrogen loading has decreased more than phosphorus loading.

Differences in anthropogenic pressure were investigated after an assumption that mean of chloride and sulphate concentrations can be used to describe the intensity of anthropogenic inputs. Anthropogenic sources of chloride are, for example, industrial chemicals, cleaning products and the use of road salts during the winter. Sulphate is often a component in fertilisers and fossil fuels. The highest concentrations of chloride and sulphate in the GML-system were found in Lake Niegocin and in other lakes close to it (Boczne, Jagodne and Szymoneckie, fig

13). This might be caused by the high volume of wastewater discharged to Lake Niegocin (14400 m^3 per day, in comparison to 3900 m^3 discharged to Lake Mikołajskie and 1300 m^3 to Lake Ryńskie, BDL 2012). Mean chloride and sulphate concentrations had a moderate positive correlation with the trophic state of lakes (fig 14). The chloride concentrations were clearly below acute toxicity levels, but still indicated a slightly increased level of salinity, which could be a chronic environmental stressor in freshwater environments (Kaushal et al. 2005). The effect of salinity in freshwaters is not well known. However, there is a trend towards increasing chloride concentrations in freshwaters globally; its effects should be investigated in the future.

Lake Niegocin received a high amount of sewage (BDL 2012). Before effective wastewater treatment, Lake Niegocin functioned as an important source of nutrients for many lakes which received water from it (at least lakes Boczne, Jagodne, Szymoneckie, Szymon and Tałtowisko). This can be seen from historical records: eutrophication and later oligotrophication of these lakes followed the trophic state changes of Lake Niegocin with a delay of a few years (Siuda et al. 2013). Even though diffuse sources might generally be more important sources of nutrients than point sources in Poland (HELCOM 2011), the importance of point sources is obvious at a local scale. The point sources are probably much more important determinants of trophic state in lakes such as Niegocin, Ryńskie and Mikołajskie, and also in lakes which receive nutrients from them.

Generally, in the GML-system, non-point sources of nutrients might be as important as point sources. Non-point sources (including agriculture) are currently the largest external source of nutrients in Poland: more than half of the pollutant load originates from diffuse sources, with less than one fourth coming from point sources (HELCOM 2011). The rest of the diffuse nutrient load originates mainly from atmospheric deposition, natural background and transboundary sources. The over-application of mineral fertilisers has been estimated to be the main cause of surplus of fertilisers on agricultural fields (Gaj and Bellaloui 2012). A surplus of fertilisers can

lead to increased leaching and runoff of nutrients to aquatic environments. At the beginning of the 1990s, the privatisation of many state owned farms, cuts in governmental subsidies for fertilisers, a decrease in the number of farm animals (20.6%) and a decrease in agricultural land area all led to a general lowering of the intensity of agricultural operations in Poland (Jankowiak et al. 2003, GUS 2012). This can be seen in the fast decline in the use of mineral fertilisers in Poland in 1990 (from 924600 tons of P and 1478600 tons of N in 1989 to 151400 tons of P and 569300 tons of N in 1991, IFA 2012). From 1991 to 2008, the consumption of fertilisers has been slowly recovering. Since 2008, the consumption appears to have stabilised at about 380 thousand tons of phosphorus and 1050 tons of nitrogen, which corresponds to about 40% of the phosphorus and 70% of nitrogen mineral fertiliser consumption before the collapse in 1990. Simultaneously with the increase in mineral fertiliser application, the surplus has also been increasing: the phosphorus surplus in 1998 was 3 kg-P ha^{-1} and in 2008 already 5 kg-P ha^{-1} (Gaj and Bellaloui 2012). According to another publication (Larsson and Granstedt 2010), the phosphorus surplus might be as high as 19 kg-P ha^{-1}.

In the GML-system, the changes around 1990 did not have a significant influence on agricultural area (SOS 1981-1996, BDL 2012), but the consumption of fertilisers did decrease to less than half of previous values (fig 12, SOS 1992-1996). The area of agricultural land decreased slightly during the 21st century (from 44.6% in 2004 to 42.9% in 2009, BDL 2012), but the usage of fertilisers almost doubled at the same time (BDL 2012, fig 12). Assuming that non-point sources are an important external source of nutrients in the GML-system (as they are in general in Poland, HELCOM 2011), external nutrient load might have increased in recent years due to the increase in fertiliser consumption. Decreased inputs from point sources (mainly municipal wastewater treatment plants) might not be able to compensate for the increased agricultural inputs.

Another potential agricultural nutrient source is manure. The potential for the leakage of nutrients can be high when inadequate methods to store manure are used (Eriksson et al. 2007). Also, in Poland, agricultural lands are still too often directly adjacent to rivers or lakes, without any protective riparian vegetation barriers. This exacerbates the run-off of agricultural nutrient pollution to surface waters.

The Masurian region is an important tourist destination in Poland. In Warmia-Masuria province, the number of accommodated tourists reaches almost one million per year, with the peak season is during July and August (BDL 2012). This means that there is a dramatic increase in local populations, especially during summer months. The wastewater treatment might become stressed during the peak tourist season (although no data are available about variations in wastewater treatment efficiency within a year) and the total load from wastewater treatment arises as the amount of people utilising the sewage network is multiplied. Thus, tourism is also probably an increasingly important source of pollution for the GML-system.

Regeneration of nutrients and microbial activity

The trophic state of an aquatic ecosystem can be defined through differences in rates of supply and loss of organic matter (Nixon 1995, 2009). This could be seen from figs 15 and 16, which showed an increase in the ratio of primary production to respiration in the GML-system when the trophic state increased. In eutrophic environments, primary production was the dominant process, which led to an accumulation of organic matter. In mesotrophic environments, primary production and respiration were more balanced (when TSI was below 50 the ratio between GPPr and Rr was about 1, figs 15 and 16) and the accumulation of organic matter did not occur.

The rate of respiration also increased in eutrophic lakes of the GML-system (fig 17), despite the fact that primary production was the dominant process. In eutrophic lakes,

decomposition rates of organic matter (measured as respiration rate, AMP and APA activities) were higher than in mesotrophic lakes. These results fit well with previously published results from the GML-system (Chróst and Siuda 2006).

Generally, differences between lakes of the GML-system in microbial heterotrophic activity (respiration rate, AMP and APA activities, fig 18) followed a similar pattern to differences in primary production (fig 19). However, only microbial activities correlated with TSI. The primary production rate of phytoplankton had a high variance between 2009, 2010 and 2011. Because of the high variance, no correlation between measurements of NPPr and TSI was found during the investigations (results not shown). The percentage of extracellular release (PER) was also variable and did not correlate with TSI; it generally remained between 15 and 30% of the primary production rate measured with C^{14}-method (fig 19).

Rate measurements of microbial activity can give important information about short-term changes within ecosystems. For example, the ratio of GPPr to Rr can be a good tool to estimate whether primary production or respiration is dominating in the ecosystem. The ratio is believed to be sensitive to seasonal changes in the formation and collapse of phytoplankton blooms and changes in leaching or flushing rates of decomposable organic matter from the surrounding terrestrial environment to the aquatic ecosystem. The sensitivity is indicated by large standard deviations (SD) shown in fig 15. Despite that, activity measurements provide a lot of useful information. For example, in the GML-system, the increase in the ratio of GPPr to Rr with trophic state shows that the functioning of the ecosystems changed with eutrophication. The primary production rate became dominant over the respiration rate, which is likely a reflection of primary and secondary producer biomasses and activities.

An important step towards a better understanding of the functional differences between oligotrophic and eutrophic ecosystems would be a method for quantifying the regeneration rates of carbon, nitrogen and phosphorus in a comparative manner. Preliminary work for the

development of such a method was performed during the present investigations (Wachowicz 2012). The preliminary results indicated a decreased ratio of regenerated nitrogen to phosphorus during eutrophication. This can be a potentially important difference in the functioning of oligotrophic and eutrophic aquatic ecosystems. The final results will be published later.

Nutrient limitation during trophic state changes

Nutrient limitation of phytoplankton biomass or rate of primary production is an important aspect of lake ecology, which is particularly interesting for lake management. To determine which factors cause an increase in the biomass of phytoplankton has been one of the main goals of hydrobiology since the very beginning. Here, the objective was to find out how nutrient limitation has been changing in the GML-system since the 1980s and which factors regulated chl *a* concentration, phytoplankton composition and biomass in the GML-system.

Large changes in the TN:TP ratio occurred in the GML-system since the 1980s (figs 20-23). They were driven by alterations in the amount and ratio of nutrient inputs to the system. The changes in the amount of external inputs of nutrients were discussed earlier, but the ratios were also modified simultaneously.

Improvement of the efficiency of wastewater treatment increases the TN:TP ratio of the treated sewage, because the improvements especially target the amount of phosphorus (not taking into account the improved nitrogen removal systems). In the GML-system, the first sewage treatment plants had only mechanical treatment. Consequently, mean TN:TP ratio in point sources to the GML-system was estimated to be 4:1 in 1974 (Giercuszkiewicz-Bajtlik and Głąbski 1981). Since the 1970s, the sewage plants have been upgraded and the mean TN:TP ratio of treated sewage has increased. Biologically treated sewage from the treatment plant of Ryn had an average TN:TP ratio of 8:1, and sewage from plant with improved nutrient removal in Mikołajskie had a mean value of 23:1 (calculated based on data from 1998-2011, BDL 2012).

The wastewater treatment plant in Giżycko discharged wastewaters with an average TN:TP ratio of 26:1 until 2002. After constructing upgrades, the amount of nitrogen decreased in the discharged wastewaters and its mean TN:TP ratio is presently 15:1.

Whether changes in fertilisation changed the TN:TP ratio of agricultural inputs to the lakes could not be estimated based on the available data; it would have required information about surplus amounts of nitrogen and phosphorus fertilisers on fields, about soil characteristics and how these factors altered leakage and runoff rates to neighbouring lakes.

The increase in TN:TP ratio of external inputs was reflected in the limnetic TN:TP ratio. Most of the available early information with a significant amount of nitrogen data was published by Kufel (2001). According to these findings, the TN:TP ratio of the GML-system during intensive eutrophication was about 15:1 (years 1988-1996, fig 20). During oligotrophication, the TN:TP ratio increased to 35:1.

Generally, nutrient limitation changed from phosphorus limitation towards nitrogen limitation on a continuum of increasing TSI (fig 23). In other words, at low trophic states, phosphorus limitation prevailed and at higher trophic states nitrogen limitation took over. A similar change in nutrient limitation at a continuum of phosphorus concentrations was found earlier, for example by Jeppesen et al. (2005). However, whether this is solely a reflection of a high amount of phosphorus inputs to eutrophic environments or of other processes within a lake ecosystem (for example denitrification) remains unknown.

The high TN:TP ratio did not remain consistent. In 2005 and 2010, low TN:TP ratios returned (mean 16:1) and nitrogen limitation was prevalent, as indicated by a correlation between chl *a* and TN (fig 21). There was no information available to estimate what environmental factors caused the strengthening of nitrogen limitation in 2005, but there were good indications about the causes in 2010 (discussed later).

In the GML-system, the amount of chl *a*, cyanobacterial biomass and number of heterocysts increased in 2010 when the mean value of TN:TP ratios was 16:1 (figs 10 and 11). Chl *a* concentration showed a close correlation with TN, indicating that phosphorus was overabundant (fig 21). In 2009 and 2011, when the TN:TP ratio was 35:1 on average, the amount of chl *a* showed co-limitation by both nitrogen and phosphorus. These results fit well with previously published results about an increase in the biomass of cyanobacteria and the number of heterocysts when nitrogen limitation becomes stronger (Smith 1986, Nõges et al. 2008, de Tezanos Pinto and Litchman 2010). According to de Tezanos Pinto and Litchman (2010), the critical value of TN:TP ratio is 32:1, which is when a sudden change in cyanobacteria biomass occurs.

Generally, chl *a* and TP concentrations in the GML-system followed a relationship established by Dillon and Rigler (1974, fig 22), which indicated that phytoplankton biomass followed TP concentrations. Despite the strong correlation between chl *a* and TN in 2010, making a conclusion that phytoplankton biomass was limited by nitrogen is problematic. It can be argued that the strong correlation between TN and chl *a* was caused by a temporary overabundance of phosphorus and that co-limitation or phosphorus limitation had already returned by the following year. Rather, these results as a whole showed a variable co-limitation.

The most undesirable effects of eutrophication and an increase in phytoplankton biomass are related to cyanobacteria blooms. The environmental factors which controlled cyanobacterial biomass were evaluated through a forward stepwise regression model using cyanobacterial biomass as a dependent variable (the model selects which variables were needed to explain most of the variation in the dependent variable, fig 24). According to the model, cyanobacterial biomass information about TSI, TN:TP ratio and biomass of algae are all needed to produce reasonably accurate predictions (table 5, figs 25 and 26). These results confirm the suggestions by multiple authors that cyanobacteria benefit from a eutrophic environment with a

low TN:TP ratio, and that strong competition with algae can also modify the abundance of cyanobacteria (Smith 1986, Dokulil and Teubner 2000, Ferber et al. 2004, Nõges et al. 2008, Vrede et al. 2009).

In fact, the inclusion of TSI to the multiple regression model is dubious, because it is not a fully independent variable in comparison to cyanobacterial biomass. TSI is calculated as a mean of indexes based on Secchi disk visibility and concentrations of TP and chl *a* (Carlson 1977). In the GML-system, chl *a* was strongly correlated with cyanobacterial biomass (results not shown). However, without the inclusion of TSI, no good model was achievable (even when including TP and Secchi disk visibility to the selectable independent variables). This likely indicates how strongly cyanobacteria benefit from the effect of shading competitive algae or from otherwise turbid conditions at higher trophic states (Smith 1986, Peretyatko et al. 2012). This is also the reason why biomass of algae was selected for the forward stepwise regression model. Despite the problem of including a dependent variable in the model, other factors were also selected as significant contributors to the model. This indicates that TSI as a single factor cannot determine the biomass or phytoplankton composition. Rather, synergistic effects of phosphorus together with nitrogen and also inside the phytoplankton community determine together the phytoplankton biomass and community composition in the GML-system.

According to the model, an appropriate strategy to avoid cyanobacterial dominance and blooms in the GML-system would be the management of the lakes, with a goal of keeping the TN:TP ratio above the critical value of 32:1, which was determined by de Tezanos Pinto and Litchman (2010). This confirms results from an ecosystem experiment performed by Schindler et al. (2008), which indicated that decreased nitrogen inputs to a lake strongly favoured nitrogen-fixing cyanobacteria. However, one of the main conclusions of Schindler et al. (2008), that cyanobacteria supplied enough nitrogen to the ecosystem to completely offset nitrogen limitation, was disputed by Scott and McCarthy (2011). Still, according to Schindler (2012), no

supporting data about nitrogen limitation of phytoplankton biomass have been found in ecosystem scale experiments, and all speculations about this topic have been based on mesocosmos or microcosmos experiments. This problem remains one of the less understood aspects of nitrogen limitation and nitrogen cycles in lakes. Consequently, it might be more appropriate to treat nutrient limitation as a complex web of interactions and co-limitations. The best way to approach the problem could be through the effects of the TN:TP ratio on phytoplankton composition and the biomass of groups of phytoplankton rather than through correlations between a nutrient and total biomass or activity of phytoplankton.

Decreasing cyanobacterial biomass could lead to multiple beneficial changes in freshwater ecosystems; for example, improved trophic transfer of energy and matter (eukaryotic algae can support higher biomass of grazers), decreased rate of sedimentation of organic matter and improved oxygen conditions in sediments (mostly in shallow lakes, Havens 2008). Could these beneficial effects be obtained simply through manipulation of the TN:TP ratio? While changes in nitrogen cycles under differing external nitrogen inputs remain poorly understood, manipulation of TN:TP ratios will be hard. Another strategy to control cyanobacterial biomass is through the influence of TSI and algal biomass. Lowering the trophic state would lead to multiple beneficial changes: generally lower phytoplankton biomass, a decreased ability of cyanobacteria to overshadow eukaryotic phytoplankton, and an increased TN:TP ratio. These changes should be sufficient to prevent cyanobacteria from being the dominating phytoplankton group and to prevent cyanobacterial blooms. Consequently, the best management strategy for temperate lakes for limiting cyanobacterial dominance and blooms might still be the classical strategy of decreasing the amount of phosphorus entering the lakes and thus decreasing the trophic state. Decreasing nitrogen inputs can, at its worst, cause an undesirable increase in cyanobacteria biomass.

Climatic influence on the trophic state of the GML-system

The aim of the chapter was to find out to what extent weather conditions influenced the trophic state of the GML-system and what caused high variability in multiple factors, such as chl *a* and TP concentrations and cyanobacterial biomass, during 2009-2011 (fig 27). In 2010, an extreme weather pattern occurred, as a strong heat wave hit the whole of Eastern Europe (Barriobedro et al. 2011, Rahmstorf and Coumou 2011). Generally, in Poland, it was associated with flooding of rivers caused by heavy rains throughout the summer. The mean flow rate of many rivers doubled and the mean yearly rainfall was about one third higher than the long-term mean from 1971-2005 (GUS 2011). However, in the Masurian region, precipitation was even lower than the mean in July and about 75-125% above the long-term means in August (when rainfall amounted to just 110-160 mm, IMGW 2010a,b). Mean air temperatures were classified as extremely warm both in July and August (mean temperature was above 21°C in July and above 19°C in August). The temperature anomaly (expressed as a difference to mean temperature from years 1971-2000) was 3-4°C in July and 2°C in August. Sunshine duration in July was also much higher than normal (about 40-80 hours more than on average during same time of year), but in August it was either within usual variation or just slightly higher (0-30 hours above mean). Thus, the weather pattern in 2010 in the GML-system followed the changes that are expected with climate change.

Changes in air temperatures influenced the temperatures of epilimnia. Mean epilimnetic temperature was 19.6°C in 2009, 23.0°C in 2010 and 20.4°C in 2011 (fig 28). Epilimnetic temperatures higher than 25°C were found in lakes Tałtowisko, Ryńskie and Tałty in 2010. The mean values in 2010 were just slightly lower than the highest water temperatures found by

Skowron (2009) in the lakes of Northern Poland from a period of 35 years (from 1971 to 2005). According to Skowron, the mean temperature of the ten hottest lakes was 23.8°C in July 1994. In the GML-system, 11 lakes had epilimnetic temperatures higher than 23°C in July 2010. This indicates that July 2010 was one of the two most extreme years by water temperature during a 40 year period in Northern Poland.

The high temperatures in 2010 caused a decrease in the thickness of the epilimnetic layer of the studied lakes (fig 29). Usually in the GML-system, the euphotic zone is distinctly shallower than the epilimnetic zone. The ratio of thickness of euphotic zone to thickness of epilimnetic zone decreased in 2010 and the ratio was close to one in multiple lakes (fig 30). This would have improved the light environment for phytoplankton, because species which are not capable of regulating their depth in the water column would experience less time in darkness i.e. outside the euphotic zone during daylight hours. However, this did not significantly influence chl *a* concentration, cyanobacterial or algal biomasses.

Changes in oxygen conditions of the profundal zone and its effect on the functioning of the GML-system were investigated. Changes in the thickness of the hypoxic zone were not statistically significant (fig 31), possibly due to large differences in the maximum depth of lakes. Still, a slight increase in thickness of the hypoxic zone was observable in many stratified lakes in 2010. For example, in Lake Tałty site 2, the thickness of OBZ (a zone between hypoxic water layer and epilimnion) was only 1.3 metres in August 2010. Usually, the thickness of OBZ ranged from 2 to 16 metres in lakes which had a hypoxic zone.

In the lakes where the thickness of OBZ decreased below 5 metres, prominent increases in the concentrations of TP, TN, DOC, chl *a*, cyanobacterial biomass and number of heterocysts were observed (fig 32). In particular, the increase in biomass of cyanobacteria was eminent (figs 10, 27 and 32). A conspicuous increase in limnetic TP concentration only occurred in lakes which simultaneously had a shallow OBZ (less than 5 metres deep) and a high profundal TP

concentration (fig 33). This indicates that phosphorus, which was released from sediments, accumulated in the hypoxic profundal zone and was only able to reach the limnetic layer when the OBZ was shallower than 5 metres. When this occurred, a strong eutrophication effect was observed in the limnetic layer (fig 32).

The limnetic TN concentration decreased in 2010 (fig 27), confirming the assumption that the eutrophication effect in the GML-system in 2010 was not caused by increases in external inputs. Otherwise, the limnetic TN concentrations would have also increased. Moreover, in July 2010, precipitation was lower than on average and only increased slightly in August 2010 (IMGW 2010a, b). Thus, it is not probable that runoff of nutrients from agricultural fields played any significant part in the ecological changes, because the eutrophication effects were already visible in July. Thus, the observed changes in nutrient concentrations were presumably a result of internal loading, but the possibility of increased external inputs cannot be completely excluded.

An increase in TP and decline in TN concentrations caused a significant drop in TN:TP ratio in 2010 in comparison to 2009 and 2011 (fig 27). Also, the biomass of algae was lower in 2010 than in 2011, possibly as a consequence of being outcompeted and shaded by cyanobacteria or due to unfavourably high temperatures and other environmental factors.

Based on the obtained results, the proliferation of cyanobacteria was influenced mainly by internal loading of phosphorus, low TN:TP ratio and weak competition by algae. Other contributing factors were long sunshine duration, high water temperatures and possibly also a stable water column. All of these factors together contributed to the intensive eutrophication effect. The results fit quite well with predictions about increasing cyanobacteria dominance during climate change (Wagner and Adrian 2009, Elliot 2012). However, some authors have assumed that total phytoplankton biomass production over a year would not change (Elliot 2012) or would even decrease if strong stratification causes a reduction of internal flux of nutrients

from the profundal zone to the epilimnion (Wagner and Adrian 2009). Based on results obtained during the present investigations, this does not seem to be the case in freshwater environments such as the GML-system (stratified eutrophic lakes with hypoxic profundal zones and known history of high external inputs). Hypoxia (Adrian et al. 2009) and internal loading (Nürnberg et al. 2012) have been predicted to intensify due to climate change. Also, recent oligotrophication is known to magnify the importance of internal loading (Nürnberg 1984). Such changes in the intensity of internal loading can be reflected in increased limnetic TP concentrations and phytoplankton biomass.

There is not enough available data to estimate which mechanism allowed phosphorus to pass the OBZ when its depth was reduced below 5 metres; it is unlikely that diffusion would be fast enough. One of the mechanisms could be heavy ship traffic, which occurs in the GML-system during summer months. The ships create strong waves, which could cause mixing of epilimnetic waters with hypoxic nutrient rich waters. Another known possible mechanism is vertical entrainment (Blanton 1973, Kamarainen et al. 2009), which is a transfer of water from the profundal zone to epilimnion, when the thickness of epilimnion increases (Blanton 1973). Such transfer of water occurred in the majority of the studied lakes in 2010 between July and August (fig 34). The calculated limnetic entrainment values were within a range reported by Blanton (1973) for the Experimental Lakes Area. The entrainment rate is known to be faster in larger lakes. Thus, possibly due to the relatively small area of lakes in the GML-system, entrainment was not found to have influenced limnetic nutrient concentrations. Entrainment can also occur as “leakage around the edge”, where the thermocline is weakened near the littoral zone, allowing the profundal waters to mix upward. In the GML-system, littoral entrainment might be a more important process than limnetic entrainment. The lakes of the GML-system have relatively small and mainly elongated shapes (table 6), which increase the importance of the littoral area for lake-wide budgets and processes. Limnetic and littoral entrainment in the GML-

system should be investigated in more detail in the future. Because of the slow entrainment rate, the thickness of OBZ was found to be a far more superior predictor of limnetic nutrient and chl *a* concentrations and phytoplankton biomass in the GML-system. The thickness of OBZ might be a generally useful parameter for predicting internal loading of phosphorus in other relatively small lakes which have hypoxic nutrient rich zones.

Kufel and Kalinowska (1997) found in Lake Mikołajskie that the metalimnion efficiently isolated epilimnion from hypolimnion during summer months and that the flux of phosphorus from hypolimnion did not reach epilimnion. However, based on data from their publication, it could be estimated that the thickness of epilimnion was between 5 and 6 metres in July and August. They found a distinct increase in soluble reactive phosphorus at depths between 10 and 12 metres, indicating that the thickness of OBZ was somewhere between 4 and 7 metres. According to the results presented here, the OBZ might have been too thick for the phosphorus to penetrate through it to epilimnion. Therefore, the results of Kufel and Kalinowska (1997) do not prove that the internal loading of phosphorus could not occur in Lake Mikołajskie when hypoxia is stronger than during their studies.

In summary, internal loading of phosphorus in stratified lakes of the GML-system seems to occur only when OBZ is thinner than 5 metres. Such conditions occurred in 2010, which was an extremely hot year. Consequently, cyanobacteria blooms induced by internal loading events can be expected to become more common in the future.

Where and how can oligotrophication be reached?

Lakes can be classified according to susceptibility of their TSI to decreased external inputs (so called sensitivity of a lake's trophic state). Indexes for this purpose have been developed (for example Reynolds 2003 and Bajkiewicz-Grabowska 2011). The sensitivity of a lake's trophic status depends on multiple factors, which include, for example, significant point

and diffused sources of nutrients, proportion of lake area with less than 5m depth, pH, water retention (Reynolds 2003), ratio between lake area and the lake's drainage basin area and the average slope of the terrain (Bajkiewicz-Grabowska 2011). Based on such classification, it is possible to identify the eutrophic status of a lake as reversible (recovery could be immediate and proportional to the decrease in phosphorus input), hysteretic (recovery requires extreme reductions in phosphorus input over a long period of time) or irreversible (recovery impossible through input decrease alone, Reynolds 2003).

Lakes of the GML-system were all classified as eutrophic during 2009-2011 (a range from 50 to 63, fig 7). Improvement of the ecological state of these lakes could lead to, for example, decreased biomass of cyanobacteria, smaller likelihood of cyanobacterial blooms, improved Secchi disk visibility and a decreased occurrence of hypoxia (Havens 2008). Analysis of sensitivity of the lakes' TSI towards external inputs would show where improvements are possible through nutrient input reductions only and where additional management measures are required.

Characteristics selected for classification were: land use in the vicinity of the lakes, presence of riparian buffer zones, significant point sources, shape of lake, lake volume, connectivity to other lakes and sulphate concentration (tables 6 and 7). The amount of characteristics used for classification here was rather small, due to a lack of available information. However, even such simple classification permitted the estimation of differences in sensitivity between lakes of the GML-system and for pointing out directions for future research.

Grading based on the selected variables correlated positively with mean TSI and explained about 55% of the variation (fig 35). By influencing some of the used variables, water quality of the GML-system could be substantially improved. The main targets are: point sources of nutrients, land use in the vicinity of lakes and vegetative riparian buffers.

One of the best ways to limit runoff of fertilisers to the lakes could be the construction of vegetative riparian buffers along lake shores (Jankowiak et al. 2003). These zones are still missing or inadequate in many southern lakes of the GML-system. Riparian buffers can be forests, shrubs or grass. A forested model by Welsch (1991) is the most commonly used and widely accepted. Vegetative riparian buffers offer both nutrient removal capacity and habitat for plants and animals. They reduce phosphorus inputs more effectively than nitrogen inputs, but the capacity for nitrogen removal can be increased if flow rate within the buffer is sufficiently slowed down and the flow of water is fully intercepted by vegetation (Mitsch et al. 1999). This increases the effectiveness of denitrification. The quality and structure of presently existing vegetative riparian buffers in the GML-system is poor and mainly accidental. They only rarely include forests (for example Lake Bełdany) and more often marshes and meadows left free from agricultural or urban management for any reason.

Other important factors, which can influence a lake's sensitivity towards nutrient load changes, are related to its morphological characteristics. Already, Vollenweider's model (1969, 1975), which predicts a lake's eutrophic status, recognised that high volume and water renewal time help with diluting nutrient inputs. The importance of diffuse inputs is smaller in lakes which have a low ratio between lake area and length of shoreline (circular lakes). When the ratio is higher (elongated lakes) the significance of inputs from agricultural fields has higher importance for the lake's nutrient balance. In the GML-system, the northern lakes all have a circular shape, while the southern lakes are more or less elongated (the most noticeable exception is Lake Śniardwy). Consequently, even after the establishment of vegetative riparian buffers, the southern part would still be more vulnerable to diffuse agricultural inputs than the northern part of the system. This could be compensated by making the riparian buffers more efficient and by reducing nutrient input from the wastewater treatment plants.

Additionally, the avoidance of over-fertilisation and runoff of nutrients from stored manure can be important factors. An independent research organisation (IEEP 2003) estimated that only 5% of Polish farmers follow government regulations about correct manure storage. It is possible that the promotion of good agricultural practices could also yield good results in general. As agricultural sources are estimated to account for more than half of nutrient inputs to waters in Poland (HELCOM 2011), there is high potential to influence the trophic state of all lakes through minimising agricultural nutrient inputs. At the moment, there is no information regarding nutrient leakage from manure and fields in the vicinity of the GML-system.

Sulphate is used in fertilisers and in industry (mining, paper mills and tanneries for example) and can also be released during the burning of fossil fuels. The highest sulphate concentrations in the GML-system can be found in lakes which do not have vegetative riparian buffers and also in Lake Niegocin (table 6, fig 36). This indicates that the majority of sulphate in the GML-system originates from anthropogenic sources such as agriculture and urban centres. High sulphate concentration can contribute towards faster internal cycles of phosphorus (Caraco et al. 1993, Katsev et al. 2006); as a result, an in-depth analysis might reveal differences in the rate of phosphorus cycling between northern and southern lakes. Also, chloride has mainly anthropogenic origins in freshwater environments (Sapek 2009). However, its influence on nutrient cycles and trophic states are not known.

Only three lakes in the GML-system received directly significant amounts of nutrients from point sources: Niegocin, Ryńskie and Mikołajskie. Wastewater treatment plants in Giżycko and Mikołajki have been equipped with increased nutrient removal, but Ryn's plant only had biological treatment (BDL 2012). According to BDL (2012), Lake Ryńskie received the highest phosphorus point source load from all of the studied lakes. Thus, the potential for influencing trophic status could be the highest in Lake Ryńskie. Upgrading the wastewater treatment plant in

Ryn from biological treatment to a tertiary treatment system would decrease the importance of the plant as a point source of nutrients.

External inputs of nutrients to both northern and southern lakes could also be reduced through termination of the usage of phosphorus-containing detergents and through improvements in the efficiency of wastewater treatment with artificial wetlands. It has been speculated that one of the reasons why Lake Niegocin was able to recover almost to mesotrophic status was the fact that sewage input from the wastewater treatment plant was first directed through a small lake called Grajewko (Siuda, oral communication). This small and shallow lake (0.4 km^3, mean depth 5 metres) trapped nutrients from the output of the sewage treatment plant and protected Lake Niegocin. Presently, the treated wastewaters flow directly to Lake Niegocin. Wetland systems are sometimes used in increasing efficiency of wastewater treatment (Verhoeven and Meuleman 1999) and for reducing agricultural nutrient inputs (Zedler 2003). The establishment of wetlands in Mikołajki and Ryn, as well as the redirection of treated wastewater output back to Lake Grajewko, could substantially improve the water quality of the southern lakes and should be one of the priorities of the management of the GML-system.

It seems that there are many possible ways to decrease the external input of nutrients to southern lakes. Based on that, most southern lakes could be classified as having a reversible eutrophic status. In the 1990s, southern lakes responded to decreased nutrient inputs quite rapidly and seemingly in proportion to the decreased phosphorus input. If the same trend continues, their further recovery could again be immediate after further decreases in external inputs and be proportional to the reduction. However, an important obstacle for oligotrophication is the prevalence of hypoxia in the southern lakes. Hypoxia is a much stronger factor in the southern than the northern lakes (fig 8), and can lead to internal loading of phosphorus under certain environmental conditions. Based on the present results, internal loading of phosphorus is not a common phenomenon in the GML-system, but its occurrence might increase in the future. Thus,

it might be important to reduce external inputs before increasing temperatures cause an increase in the occurrence of internal loading events. Internal loading can also severely hinder oligotrophication in stratified lakes (Mehner et al. 2008).

If all of the proposed measures were to be implemented in the southern lakes, almost the whole southern part could become closer to the mean TSI which exists currently in the northern part (mean TSI about 52). The basic structural or functional differences between the northern and southern parts that were identified included: size and shape lakes, intensity of anthropogenic input and dimensions of indirect drainage area (for example Lake Śniardwy collects waters from a much larger area than Lake Mamry). Consequently, there is no logical reason for why TSI and other ecological factors of southern lakes could not be brought closer to the level characterising northern lakes.

The trophic state of the northern lakes could be classified as hysteretic, because oligotrophication could probably only be achieved through much stricter measures over a longer period of time. Presently, the importance of Giżycko as a point source of nutrients for the northern lakes is not fully known and should be estimated. If nutrient rich waters from Giżycko's sewage plant do not reach the northern lakes, the main sources of nutrients are diffuse sources. However, the vegetative riparian buffers, which fully cover the shores of the northern lakes, reduce the amount of diffuse inputs.

Usage of such a classification system directs attention to the characteristics which are important for management of lakes. It can be a very useful tool for ecosystem managers and researchers. Here, for example, the need for constructing vegetative riparian buffers around the southern lakes arose as a result of looking for differences between the lakes of the GML-system. Other important factors can possibly still be found, if more variables are analysed and more information is gathered.

Other factors and suggestions for future research

Internal loading of phosphorus has been shown to occur when phytoplankton is recruited from resting stages from sediments to the water column (Pettersson et al. 1993). Inside the phytoplankton cells, high phosphorus content is carried, which is acquired from sediments. At least one species of cyanobacteria, *Gloeotrichia echinulata*, has been verified in the literature as an effective phosphorus carrier. *G. echinulata* was found in lakes Łabap (in July 2010 as dominant species, biomass 9.4 mg L^{-1}) and Tałty site 1 (in August 2010, 2.6 mg L^{-1}, results not shown), but it was not found in any of the studied lakes in 2011. This type of internal loading can also happen at shallow areas of deep lakes and it does not require strong hypoxia as a precondition. Consequently, in some lakes, such a pathway can influence the nutrient budget. Its importance in the GML-system should therefore be evaluated.

Since this study has focused mainly on abiotic factors, further studies should be directed at biotic factors, which can also have a great influence on lake ecosystems. The importance of higher trophic levels (crustaceans and fish), grazing pressure and trophic structure in the GML-system should be more intensively studied. There are still not enough data to properly estimate their significance and all routes of influence. One of the factors influencing trophic state seems to be piscivores. In the GML-system, fish stocks are supplemented with restocking, which has been increasing since the turn of the century (Mickiewicz 2008). Many of the restocked fishes are predatory fishes such as pikes (*Esox lucius*). Consequently, the stocking practice may "artificially" upkeep grazing pressure on algae and thus act as a kind of biomanipulation. Other biotic factors that can modify the trophic state of a system are colonies of cormorants (*Phalacrocorax carbo*). In recent years, there have been signs of an increasing amount of colonies of cormorants in the GML-system. In theory, these birds can create oligotrophication in

the lakes where they go fishing and eutrophication at the lakes where they nest. Presently, however, their role is poorly elucidated.

Based on studies performed on the Northwest Ontario Lake Size Series, it is known that physical properties such as lake size (Guildford et al. 1994) and thickness of epilimnion (Fee et al. 1996) can be significant factors in internal nutrient cycling. In large lakes, the regeneration of nutrients in the water column seems to be more efficient and also phytoplankton seem to be less limited by nutrients than in smaller lakes. Reasons behind these phenomena are unclear. Such physical properties might influence differences in the rate of nutrient cycling also between the lakes of GML-system, and could be a potential direction of research in the future.

Multiple questions which should be addressed in future research have been raised by the results of the present investigations. Land use, vegetative riparian buffers and phosphorus dynamics of the GML-system should be still more thoroughly studied. External nutrient loading and water quality response of the GML-system could be approached through the application of Vollenweider's (1968) model or one of its updated versions (for example Jones and Lee 1986). This would give a better perspective on the nutrient budget of the GML-system. Also, the internal stores of nutrients in sediments should be analysed.

Intensification of hypoxia and internal loading during climate change are serious threats which should be further investigated; their prevention should be included in the management of the system. It should be pointed out that the decrease in nitrogen concentrations in 2010 and the lack of return to more normal values in 2011 are still unexplained. A possible explanation is that denitrification or anammox processes removed nitrogen from the ecosystem. However, how these processes influence the overall nutrient budget and the functioning of the GML-system are currently unknown.

The fast changes in 2010 might be a sign of the decreased ability of the ecosystem to resist outside influences. This might increase the vulnerability of the system to climatic

fluctuations and lead to fast changes in physico-chemical parameters in the future and can also increase the competitive advantage of opportunistic species. Consequently, community structure and biodiversity might be affected, as suggested by Pomati et al. (2012). Also, Huber et al. (2012) pointed out that different species of cyanobacteria might respond to heatwaves in a different manner. *Anabaena* sp. seemed to benefit directly from a stable water column and *Planktothrix* sp. from internal loading. Although climate change is known to benefit cyanobacteria (Elliot 2012), it seems that the benefit will not be equal to all species and thus, the species composition of cyanobacterial assemblages can also change.

Conclusions

During the 1990s, the beginning of oligotrophication was induced by simultaneous decreases of nutrient loading from wastewater treatment plants, fertilisers and detergents which contained phosphorus. Oligotrophication proceeded during 1995-2005. Since 2005, however, the progress seems to have stopped and in some cases has been even reversed. Major causes behind the cease of oligotrophication and threats to future oligotrophication are increased inputs of nutrients from agriculture, tourism and fast urbanisation. Intensification of internal loading and increases in cyanobacterial biomass are also potentially dangerous in the future, as shown by the analysis of an extreme weather event in 2010.

All lakes of the GML-system were classified as eutrophic based on data from 2009-2011. The large gradient of eutrophication from oligo-mesotrophy to hypereutrophy used to be characteristic for the lake system, but it has now practically disappeared.

It was found that the biomass of cyanobacteria can be predicted on the basis of TSI, TN:TP ratio and algal biomass. This indicates that the biomass of cyanobacteria could be regulated through these factors. As the manipulation of algal biomass and TN:TP ratio in lake water involves too many factors which are not fully understood and can be problematic in

practice, the best way to limit cyanobacterial blooms in the GML-system is the reduction of the trophic state.

In the majority of southern lakes, a significant increase in limnetic TP concentrations during spring overturn, the occurrence of hypoxia in the profundal layer during summer months and higher than 200 µg L^{-1} profundal TP concentrations indicate that there are high amounts of nutrients stored in the sediments. The stored nutrients can be returned to the water column during spring overturn and under low redox conditions during summer stratification. A thick OBZ usually separated the limnetic layer from the nutrient rich hypoxic profundal layer. However, when the buffer was simultaneously shallower than 5 metres and a high profundal TP concentration occurred, a conspicuous increase in limnetic TP concentration was observed. This indicated that for effective isolation of limnetic layer from nutrient rich profundal water, the OBZ needed to be thicker than 5 metres.

The heatwave in 2010 strongly changed the physico-chemical structure of the GML-system which led to strong proliferation of cyanobacteria and possible internal loading. If the changes which are predicted to occur with climate change occur, the GML-system will be under the threat of accelerated eutrophication even if external nutrient inputs remain at the present level.

External nutrient inputs to the GML-system can be decreased through the construction of vegetative riparian buffers and wetlands. Also, upgrading the wastewater treatment plant in Ryn would lead to significant decreases in the discharge of nutrients to Lake Ryńskie.

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